INVESTIGATING FRACTAL ANALYSIS OF ANIMAL BEHAVIOUR AS AN INDICATOR OF STRESS

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PhD

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2003

Currently, measures of on-going behaviour used in welfare assessment are commonly limited to durations and frequencies; however, these provide only a partial description of behaviour. Extracting additional information from behavioural sequences could benefit animal welfare assessment. Fractal analysis is an emerging behavioural analysis methodology that could extract such information. It calculates the autocorrelation structure of complex processes, providing a measure of the randomness (complexity) of a time series. Previous applications of the method to animal behaviour have identified changes in behaviour putatively related to health and well-being. The thesis examined whether, when applied to behavioural sequences, fractal measures could have diagnostic value in assessments of stress in farm animals. The fractal analysis methodology of Detrended Fluctuation Analysis (DFA) was applied to continuous focal observations of hens and instantaneous scan-sample observations of growing pigs.

In experiment one, the complexity of vigilance behaviour increased in young hens; both during the acute stress of open field exposure or following a five-minute period of restraint. The total duration of vigilance was increased in the open field but not following restraint. The DFA measures therefore revealed an alteration in behavioural organisation under stress not identified during standard analysis. In experiment two, a chronic intermittent stressor regime was applied to adult hens. This stressor regime caused alterations in food intake, body weight and egg production, which suggested the birds were transiently stressed. However, the behaviour of the treatment group did not differ from controls at any time point, either when using a standard analysis or a DFA. In the third experiment, a stressor regime involving repeated social defeats and additional mild stressors was applied to growing pigs. Following this regime the treatment group had higher levels of average 24hr cortisol than controls. The DFA did identify behavioural differences between treatment and controls groups; however, it is unclear if these were directly related to the stressor treatment.

The data sets generated in the experiments were used to further investigate the DFA method. Analyses showed that alterations in the duration of observation and the frequency of behavioural sampling can affect the end result. Although the analysis has some limitations it allowed novel dimensions of behavioural organization - not identified during standard analysis - to be measured. These

dimensions were independent of total durations of behaviour and they were sensitive to stressful stimuli in some circumstances. In conclusion, fractal analysis of behaviour shows promise as a tool for measuring stress but further validation is required.

Firstly, I would like to express my sincere thanks to the Universities Federation for Animal Welfare (UFAW) for funding myself and the research. I hope that they come to view it as a good investment.

I would like to sincerely thank Marie Haskell for her supervision of the project. Marie put a lot of effort into guiding me through the PhD experience. She found a balance between guidance and letting me learn from my mistakes that I hope has made me a better scientist in the process. Thanks are also due to Alistair Lawrence, Chris Glasbey, Bryan Jones and John Deag for their co-supervision of the project.

The Association for the Study of Animal Behaviour and British Poultry Science provided funds to allow me to present parts of this work at two conferences.

I would like to thank the following people for various 'helping hands' during the experimental work: Nancy Coerse, Anita Rennie, Nia Ball, Christine Ruschak, Richard Hunter, Dorothy McKeegan, Caroline Channing, and Christine Moinard. More specifically, I would like to thank: Dave Allcroft for advice on Fortran programming; Graeme Robertson for his advice on creating the blood slides and identifying the different leucocytes; Ailsa Carlisle for help with the corticosterone assay; Bob Fleming for access to the materials tester and advise on its use and Alan Thain at SAC's laboratory in Aberdeen for analysing the cortisol samples. Margaret Murray and Kenny Miller provided assistance with animal care at the Roslin Institute. All the staff at SAC's Easter Howgate pig unit (Sheena Calvert, Peter Finnie, Phil O'Neil, Joan Chirnside and Julie Stevenson) deserve thanks. I would like to especially thank Sheena for making sure everything ran as smoothly as was humanly possible. Kate Still also provided a lot of help at the pig unit while doing her thesis work.

Finally, I would like to thank Yasmin. For everything.

To my parents.

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Rutherford, K. M. D., Haskell, M. J., Glasbey, C., Jones, R. B. & Lawrence, A. B. In Press. Fractal analysis of animal behaviour as an indicator of animal welfare. *Animal Welfare*

Chapter One

Introduction

1-1. Introduction

Since the appearance, in 1964, of Ruth Harrison's book "Animal Machines" and the subsequent report into "The welfare of animals kept under intensive husbandry systems" (Command paper 2836, 1965), there has been a growing recognition that farmed animals may suffer in modern intensive systems. Increasingly, this putative suffering has been the subject of scientific research to assess its basis and potential amelioration.

In some cases, the suffering of animals in agricultural systems may be obvious. For instance, a high proportion of laying hens with skeletal fractures or lame dairy cattle provides a readily appreciable and uncontroversial measure of poor welfare on a farm, or in a system as a whole. More problematic, however, are considerations of the longer-term effects of living in poor quality environments, where the potential negative effects may be more subtle and the indicators of these effects controversial or difficult to interpret. In these circumstances, where poor welfare is suspected but not so readily identifiable, there is a need for careful measurement of various aspects of biological functioning to determine whether welfare is affected. Animal welfare science encompasses assessment of animal functioning in numerous different areas, e.g. behavioural, physiological, immunological and neurobiological. In this thesis, a new measure of behavioural function is investigated.

1-2. What is animal welfare and can it be measured?

1-2-1. Definitions of animal welfare

There has been variation in what different authors consider animal welfare to refer to. Some take a very physical and functional view and regard welfare as equivalent to a lack of disease and normal physical functioning. Broom is typically put forward as a proponent of this view. Broom's often quoted definition of animal welfare is: "The welfare of an individual is its state as regards its attempts to cope with its environment" (Broom 1986). Others have taken a more cognitive view and proposed that ultimately what matters is how an animal feels and whether it suffers (Dawkins 1990; Duncan & Petherick 1991; Duncan 1996).

The distance between these two views is often portrayed as being larger than it really is. Recently, Dawkins (2003) has proposed that the welfare status of an animal

can be simply summed up into two questions. The first is: "is the animal healthy?" The second is: "does it have what it wants?" Broom emphasises that his view does not merely concentrate on functioning and that feelings are considered as part of the coping success of the animal (Broom 1998). Similarly, Webster simply defines welfare as "the capacity of an animal to sustain physical fitness and avoid mental suffering" (Webster 1998). Good welfare might, therefore, be considered as synonymous with what the World Health Organization defines[1] as health for the human population: "a state of complete physical, mental and social well-being, and not merely the absence of disease or infirmity". This definition recognizes the fact that while there can be little doubt that disease will likely cause suffering, physically healthy individuals can still suffer.

1-2-2. The five freedoms

One key concept in animal welfare circles has been that of the 'five freedoms'. This term was originally used to refer to five different behavioural freedoms, where every animal should be free to "stand up, sit down, groom itself, turn around and stretch its limbs" (Command paper 2836, 1965). Subsequently, the term was adopted by the Farm Animal Welfare Council (FAWC) to describe the basic requirements of a farming system (Webster 1998). In FAWC's definition, animals should have:

1. **Freedom from thirst, hunger and malnutrition** – by ready access to fresh water and a diet to maintain full health and vigour.
2. **Freedom from discomfort** – by providing a suitable environment including shelter and a comfortable resting area.
3. **Freedom from pain, injury and disease** – by prevention or rapid diagnosis and treatment.
4. **Freedom to express normal behaviour** – by providing sufficient space, proper facilities and company of the animal's own kind.
5. **Freedom from fear and distress** – by ensuring conditions which avoid mental suffering.

[1] Preamble to the Constitution of the World Health Organization as adopted by the International Health Conference, New York, 19-22 June, 1946; signed on 22 July 1946 by the representatives of 61 States (Official Records of the World Health Organization, no. 2, p. 100) and entered into force on 7 April 1948

Webster (1998) notes that in practice the five freedoms represent an unachievable ideal. Indeed, it is probably the case that only a tiny percentage of the world's human population come close to fulfilling all these requirements fully.

Alternative housing systems may score more or less highly on each of the different freedoms. For instance, battery cages for laying hens, although severely restricting behavioural freedom, have the benefit of reducing disease levels. Alternatively, free-range systems provide greater opportunities for behavioural freedom but may have higher disease levels. The problem for welfare assessment then becomes one of deciding the weighting that different pros and cons should have. Is behavioural freedom more important than good health for a chicken? A veterinarian might say no, while an ethologist might say yes. Ultimately, such a decision is based on the bias of the individual rather than a thorough assessment of the relative priorities that animals might have.

So although the five freedoms provide a useful outline of the factors involved in good welfare the relative emphasis that is placed on each of the components is a subjective decision. The only way to clearly assess how the different freedoms should be balanced is to take measures of biological functioning. Such animal based measures (Whay et al. 2003) are the end result of an integration of the effects of the physical and social environment and all the experiences that the animal has in that environment, as well as the degree of immune challenge that the animal faces. In terms of these measures, the useful distinction between indicator variables and causal variables (Fayers & Hand 2002) can be borrowed from the human quality of life literature. Causal variables are those that directly contribute to the animal's welfare state. For instance, in lamb castration studies (e.g. Kent et al. 2000), the size and severity of the scrotal lesion can be measured as a causal variable. Alternatively, indicator variables are those measures that do not directly contribute to the welfare state but correlate with it. In some cases the line between what might be considered as an indicator variable and what might be considered a causal variable is blurred. Indeed, in situations involving social interaction a behaviour that is an indicator of poor welfare in the actor might be a cause of poor welfare in the recipient. For instance, frustration-induced aggression in chickens (Haskell et al. 2000) or belly nosing in pigs (Gardner et al. 2001) might be seen as an indicator of poor welfare in the actors and a cause of poor welfare in the recipients. Some measurements of

immune function also sit between the two – a compromised immune system can result from poor welfare and can in turn cause welfare to be further decreased, as the animal is left with an increased susceptibility to infection.

1-2-3. Assessing animal welfare

Fundamentally, concern for animal welfare relates to psychological factors. The principal disagreement between different researchers is mainly to do with what parameters can be used as welfare indicators. Ultimately, we cannot measure welfare as it is an internal property of the individual's conscious experience. However, it is possible to assess welfare by measuring parameters within the areas of behaviour, physiology, neurophysiology, disease, and physical factors. Each of these parameters has advantages and disadvantages. It is becoming more apparent that integrating the different approaches provides a more global and thorough assessment of an animal's welfare than single parameter measurement (Webster 1998). It is equally important that the biological basis of each of the measures used and how they relate to each other is fully understood before they are used as welfare indicators (Rushen 2003).

1-3. Thesis - Background

Behavioural analysis is important in animal welfare assessment (Mench & Mason 1997; Dawkins 1999; Rushen 2000). Measurements of behaviour provide information about an animal's state that can be compared with physiological or other measures to create an overview of biological functioning. Behavioural assessment, however, has some additional benefits beyond those of physiological measures. Principal amongst these benefits is the fact that behavioral analysis is non-invasive and potentially non-intrusive (Dawkins 2003). This allows repeated assessments of animals, with little or no disturbance to the animal (if the research is carried out carefully). Behavioural analysis is also more readily applicable in on-farm or field studies, where physiological sampling may not be possible for a variety of reasons.

Broadly speaking, the use of behavioural analysis in welfare assessment falls into two categories; measurement of on-going behaviour and behavioural tests. The latter category would include open field tests and similar 'behavioural assays', as well as preference testing and consumer demand studies. The former category

involves analysis of what animals do in their normal environments or following certain husbandry interventions. This analysis can have the aim of assessing to what extent animals can fulfil their behavioural requirements. For instance, the inability of hens to properly dustbathe in certain housing systems might be considered as a causal indication of poor welfare. Alternatively, analysis of on-going behaviour can have the aim of identifying particular behavioural parameters that can be used as indicators of poor welfare. It is this use of behavioural analysis in welfare assessment that is pursued here.

Unlike most other studies of this sort, where the aim is to identify changes in the duration or frequency of either normal or abnormal behaviours, here the aim is to study the temporal organisation of behaviour using fractal analysis techniques. These methods will be described in more detail in Chapter Three. Briefly, fractal measures of behaviour have been shown to provide useful information about either spatial or temporal patterns of behaviour that can differ from more standard summary measures. Based on previous work the project set out to investigate whether fractal analysis of behaviour patterns might have a role to play in animal welfare assessment. Specifically, the aim of the project was to investigate the validity and utility of fractal measurements of behaviour patterns as an indicator of stress in chickens and pigs.

1-4. Thesis - Outline

The thesis is divided into eight chapters.

Chapter Two: reviews the literature on assessment of animal stress.

Chapter Three: reviews the concept of fractal analysis and particularly its use in animal behaviour.

Chapter Four: describes an experiment examining the responses of young laying hens to mild acute stressors.

Chapter Five: describes an experiment examining the responses of year old laying hen to a chronic intermittent stress regime.

Chapter Six: describes an experiment in which growing pigs were exposed to a repeated stressor treatment involving both social and environmental stressors.

Chapter Seven: examines various practical details of the DFA methodology.

Chapter Eight: contains a general discussion and conclusions.

Chapter Two

Animal stress: a brief review

2-1. Introduction

The concept of animal stress is central to animal welfare science. Assessments of stress are crucial to our understanding of how animal welfare is affected by housing, husbandry and interactions with humans. This chapter briefly reviews the concept of animal stress. Firstly, the question "what is stress?" is posed. The measurement of acute stress responses is then discussed. The fact that normally adaptive stress responses can at some point become harmful (McEwen 2000b) is highlighted and the potentially deleterious consequences of stress responses are also reviewed. A final section looks at the potential for chronic stress to cause negative psychological consequences, in the form of depression, in pigs

2-2. What is stress?

The term stress is widely used yet often poorly defined. As Selye (1973) put it: "everybody knows what stress is and nobody knows what it is". Although there has been no clear consensus on a definition, stress is generally seen as the result of a challenge to an individual's homeostasis. It is important to distinguish between the stimulus (the **stressor**) that is perceived as a threat, the **stress state** of the animal and the resulting **stress response** (Dhabhar & McEwen 2001). In this schema, the stress state is viewed as involving a negative mental experience (the perception of stress, sometimes called **distress**), which is not observable and can only be experienced by the individual. In welfare research the aim is to measure aspects of the stress response as a correlate of the stress state.

A key part of that description is that stimuli need only be perceived as threatening for them to provoke a stress response. While extremes of stimulation will always result in a stress state, less extreme stimulation may also trigger a response depending on an animal's individual perception of a situation. A discrepancy between an animal's expectation and what actually happens may cause a stress response even if there is no real threat (Fuchs et al. 2001). This means that stress states and the resulting responses can be triggered by purely psychological stimuli. These 'imaginary' stressors are just as important a determinant of poor welfare as real stressors.

The perception of what might be threatening can be altered if an animal is in a state of increased fear or anxiety, or generally in animals with the trait of increased

fearfulness (Jones 1996). The predictability and controllability of a stimulus are particularly important in determining whether it is viewed as a stressor by the animal (Wiepkema & Koolhaas 1993). The psychological component of stress is well illustrated in classic experiments by Weiss (1972). In these experiments the degree of gastric ulceration in rats that were given a signal prior to an electric shock was less than in rats that were given the shocks without any signal. The physical stressors imposed upon these two groups of rats were the same; it was the psychological benefits of predicting the stimuli that reduced the harmful consequences of the stress.

One common issue in stress assessment is the degree of specificity shown by stress responses. Selye (1936) described a generalised non-specific response to potentially harmful stimuli that he called the 'general adaptation syndrome'. Perhaps the major revision of Selye's concept in the following decades has been the rejection of the idea of the stress response as non-specific (Mason 1971). Mason pointed out the incompatibility of a non-specific response and homeostasis; specific challenges to homeostasis require specific responses to allow a return to optimum conditions. Since Selye concentrated on the hypothalamic-pituitary-adrenocortical axis his concept of a non-specific response is not surprising, given that activity of this system is primarily related to psychological/emotional factors (Dantzer et al. 1983).

Early life experience can have a large and sometimes permanent influence on an individual's stress reactivity. Studies show that the stress response of an adult may be greatly affected by their treatment as neonates (Winberg 1998; Wellberg & Seckl 2001). Even prior to birth, adult stress responsivity may be influenced and altered (Braastad 1998; Bertram & Hanson 2002). Prenatal or neonatal stress can result in offspring that show increased responses to acute challenge (Edwards & Burnham 2001). Such an increase in stress reactivity could be explained using the 'smoke alarm' principle (Nesse 2000). This principle applies to biological defences where it is more costly to under-react than to over-react. For instance, if a feeding animal over-reacts to a perceived predation threat then it may lose its meal, while if it under-reacts to a genuine threat it could lose its life. The presence of early stress may be a form of biological signal to the animal that it is going to grow up in a

threatening environment, resulting in an adaptive increase in response sensitivity/reactivity (Gilbert 1998).

2-3. Acute stress responses

2-3-1. Overview

The measurement of acute responses to challenge is a common method of assessing animal welfare. Such measurement allows the aversive nature of certain acute events to be assessed and also allows any change in reactivity caused by the on-going experiences of the animal to be determined (Boissy et al. 2001). The presumption is that larger deviations from normality indicate a more stressful experience for the animal. The main categories of response that have been identified are behavioural, autonomic, neuroendocrine and immunological (Fig. 2-1).

2-3-2. Behavioural responses to acute stress

The initial response to an acute stressor is behavioural. The very first response may be a startle reaction (Broom & Johnson 1993). Commonly, initial responses will be strongly species- and stressor-specific (Rushen 2000; Blanchard et al. 2001a). In prey species, responses may relate to the adaptive anti-predator behavioural repertoire. Where there is an identifiable stimulus in the environment, the initial behavioural response may either be attempted withdrawal or a more active response, which can involve attack. In most cases, acute responses also involve heightened vigilance and attention focussed on the source of the stress (Broom & Johnson 1993; Krebs et al. 1997) and vocalisations may be heard.

Stress can be viewed as a motivational state (Jensen & Toates 1997), in that it causes the animal to change its behavioural priorities to attempt to decrease the discrepancy between its ideal conditions and reality (Wiepkema 1983). Due to this, the form of the behavioural response will be specific to remedying particular individual problems (Rushen 2000). Captive conditions may create stressors that are outwith those an animal would naturally meet. In these cases there may be no natural strategy.

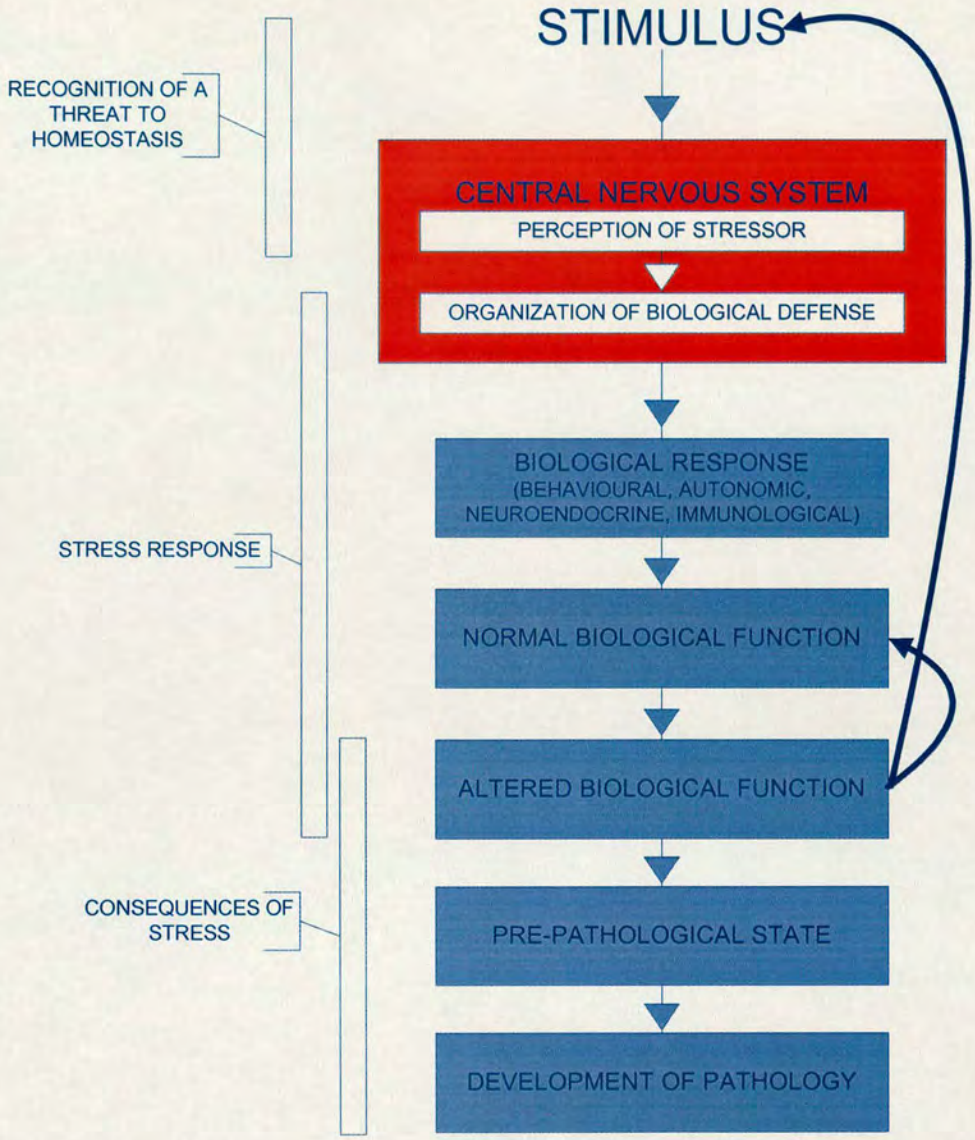


Figure 2-1: Organisation of the biological stress response

In this model (Moberg 2000), stimuli in the environment that putatively threaten homeostasis are perceived as stressors. Variability in which stimuli are perceived as threatening and the magnitude of the resulting defence response can be related to the individual's genetic background, early environment and past experience. The response can take four main forms: behavioural, autonomic, neuroendocrine and immunological. Alterations in functioning within these systems are designed to remedy the threat to homeostasis. If these acute stress responses are unsuccessful, or if they are blocked, the response may have a deleterious effect on the individual creating a 'pre-pathological' state, where animals are more susceptible to further challenge. If further challenge arises, or if the initial problem remains, pathologies may develop. Although in Moberg's model these pathologies are physical in a nature, psychological pathologies are equally possible (McEwen 2000a).

2-3-3. Autonomic nervous system responses to acute stress

On a similar timescale to the initial behavioural responses are the shorter-term components of the physiological stress response, sometimes called 'fight or flight' responses. These include increases in heart rate, respiration rate and body temperature (Broom & Johnson 1993), principally caused by catecholamine release, as a result of sympathetic nervous and adreno-medullary hormonal activity. Such autonomic measures are very sensitive to external stimuli and can be transient, so they can be difficult to measure accurately. The short latency to a response means that experimenters may be partly measuring responses to the sampling procedure itself. Autonomic responses may also show 'ceiling' effects that limit their use in the assessment of acute stress. For instance, Glatz and Lunam (1994) studied the change in heart rate in chickens during beak trimming compared to that during handling alone and found no difference between the two. Heart rate increase appears only to be a sensitive measure of mildly stressful stimuli (Wooley & Gentle 1987), so the restraint during handling without beak trimming probably caused a maximal response.

2-3-4. Hypothalamic-pituitary-adrenocortical responses to acute stress

The hypothalamic-pituitary-adrenocortical (HPA; Fig. 2-2) axis is an important body system. It is involved in the day to day regulation of numerous biological systems (including metabolism and immune function) and shows increased activity during times of stress. The axis involves an endocrine cascade that starts with the release of corticotrophin releasing hormone (CRH) from the paraventricular nucleus (PVN) within the hypothalamus. Some authors may refer to the axis as the Limbic-Hypothalamo-Pituitary-Adrenal axis (Newport & Nemeroff 2001), reflecting the involvement of limbic structures in the brain, such as the amygdala and the hippocampus, in signalling to the PVN. The release of CRH in turn triggers the release of adrenocorticotrophic hormone (ACTH) from the pituitary. This in turn causes the release of glucocorticoids (GC; predominantly cortisol in humans and pigs and corticosterone in rats and chickens) from the adrenal cortex into the blood stream.

GCs act on two different cell receptor types, the mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) (de Kloet et al. 1998; de Rijk et al. 2001). As

well as mediating the bodily functions of GCs, these receptors are involved in a negative feedback loop where GCs reduce their own production via the hippocampus, hypothalamus, and pituitary. The MR has a much higher affinity for GC, so during stress free periods negative feedback regulation occurs through these receptors (de Kloet et al. 1998; Edwards & Burnham 2001). However, when a stressor provokes an increase in circulating GCs the MRs quickly become saturated and GCs bind to GRs. The GRs therefore regulate feedback during times of acute stress.

The physiological effects of GCs are brought about by alterations in gene expression, which may take hours to days (Gold et al. 2001) and also by non-genomic effects that can be much quicker (Borski 2000). The degree of biological activity that a particular concentration of GC has is dependent on many different factors. The corticosteroid-binding globulin (CBG) protein binds to GC molecules, making them biologically inactive. CBG levels can vary and their concentration modulates the effect that a particular concentration of GC will have (Breuner & Orchinik 2002). Certain enzymes, such as 11β-hydroxysteroid dehydrogenase (11β-HSD), act to convert GCs into an inactive form and their concentration therefore also affects the impact that GC has on biological function (Seckl 1997; Sandeep & Walker 2001). Another major influence on the consequences of GC release is the absolute and relative number of MRs and GRs available, which can vary (de Kloet et al. 1998; de Rijk et al. 2001). Finally, some substances in the body may have anti-glucocorticoid actions and these influence the effect that GC has in the body. For instance, dehydroepiandrosterone (DHEA) opposes GC functions in metabolism, stress physiology and immunity (Wolf & Kirschbaum 1999; Hu et al. 2000; van Broekhoven & Verkes 2003). An alteration in the circulating level of DHEA can therefore affect the magnitude of effect that a particular concentration of GC will have (Goodyer et al. 2001).

One potential problem with using HPA activity as a measure of acute stress is that increased HPA activity can occur as a result of events that are neutral or positive from a welfare point of view (Dawkins 1999). For instance, oviposition in laying hens is associated with an increase in HPA activity that occurs even when hens are provided with an artificial nest (Beuving & Vonder 1977, 1981). In pigs, sexual activity can increase HPA activation (Levis et al. 1995). These studies reflect

the fact that a certain degree of HPA activity is desirable and necessary for normal life. Although it is clear that over stimulation can result in stress it is also the case that under stimulation can cause decreased welfare (Zulkifli & Siegel 1995). For instance, low levels of stimulation may cause boredom (Wemelsfelder 1993) and may also impair an animal's ability to deal with potentially stressful situations (Jones 1996). There are also physiological requirements for a certain level of HPA activity. During illness, excessively low levels of HPA output – corticosteroid insufficiency – can be a threat to health (Cooper & Stewart 2003).

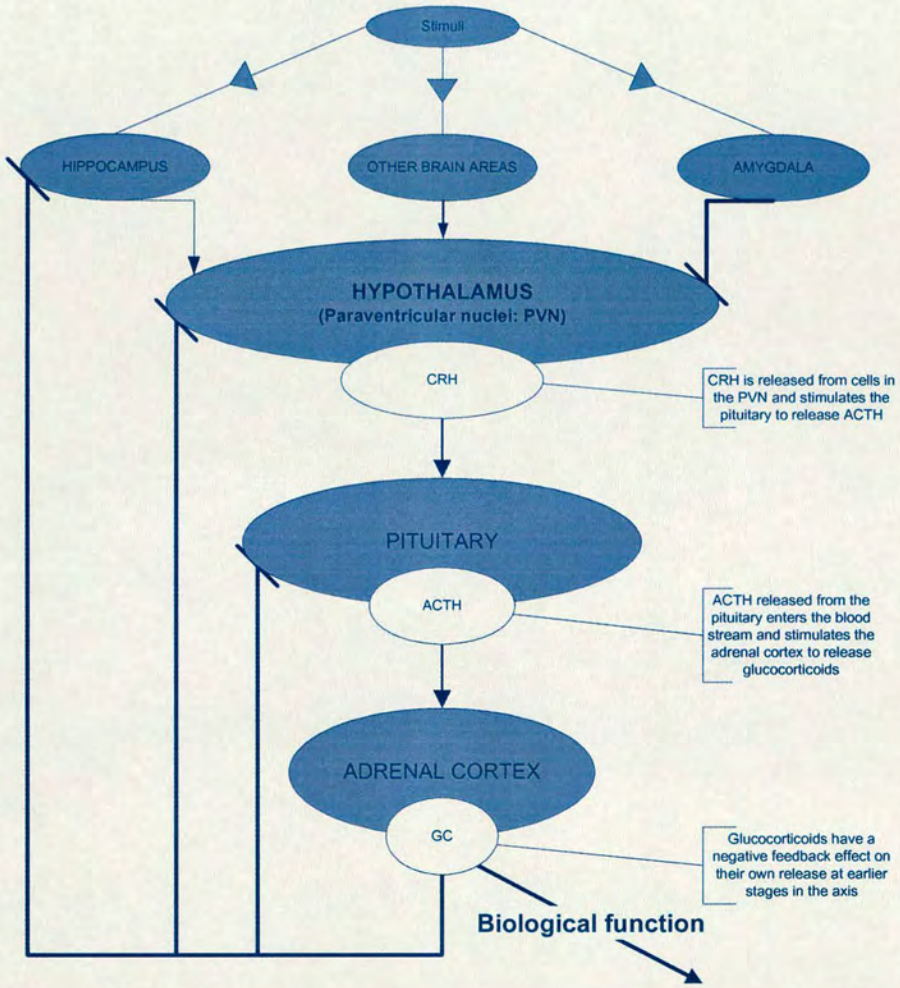


Figure 2-2: The hypothalamo-pituitary-adrenocortical axis

Details of the axis and abbreviations are described in the text.

2-3-5. Immunological responses to acute stress

The interaction between neuroendocrine systems and immune function is becomingly increasingly well described (Marsh 1992; Besedovsky & DelRey 1996; McEwen et al. 1997). Although stress is most commonly considered to be immunosuppressive, mild or short-lived stress may actually enhance certain aspects of immunity. From an evolutionary viewpoint it makes little sense for all stress states to adversely affect immunity, as typically acute stress states would occur at times when the risk of injury was increased. Rather, some aspects of immunity should be enhanced during brief acute stress, as the immune system prepares for challenge. Dhabhar and McEwen (1997, 1999) have shown that acute stress does indeed enhance skin immunity. However, it is the long-term consequences of stress for the immune system that has received the most attention in welfare research (section 2-4-3).

2-4. The chronic consequences of stress

2-4-1. Overview

Acute stress responses are transient and help the animal to adaptively deal with a challenge (Wiepkema & Koolhaas 1993). However, in captive conditions normally adaptive phenomena can become maladaptive and lead to decreased welfare. This can be because responses are triggered at an increased frequency, or because responses are ineffective in dealing with the challenges imposed. Equally, the captive environment can prove to be a source of repeated acute stressors and can create anxious animals that perceive danger where it does not exist (Rosen & Schulkin 1998). An individual's capacity to cope with a stressor challenge is dependent on its previous experience and in the generally bland housing environment of industrial agriculture this experience may be extremely limited. In some cases the captive environment may also be a source of continuous stress (e.g. environmental parameters or crowding). In these cases, when responses are repeatedly triggered, or do not remedy the situation or where stressors in the environment are continuously present, a long-term state of chronic stress may emerge.

In Broom's often-quoted definition of welfare what is important is the animal's success at coping with the stressors it faces (Broom 1986, 1996). It is important to be aware, however, that apparently successful coping may come at a cost. Situations that chronically or repeatedly stress an animal are energetically costly (e.g. Laugero & Moberg 2000ab). When multiple stressors exist these can have additive or synergistic effects (McFarlane et al. 1989ab, McFarlane & Curtis 1989, Hyun et al. 1997). Over the long-term, stress causes a shift in allocation of resources from activities such as growth and reproduction to simple maintenance processes (Siegel & Gross 2000). In some cases, animals may exist in a state of 'sub-clinical stress' where the biological cost does not obviously impinge on functioning (Moberg 2000). In such a state, animals may be under an increased risk for pathology should they be challenged with another stressor (Elsasser et al. 2000; Moberg 2000).

In stress research such a biological cost has been called an allostatic load (AL) (McEwen & Stellar 1993; McEwen & Wingfield 2003); a concept developed from Sterling and Eyer's (1988) introduction of the term 'allostasis'. Allostasis is defined as 'stability through change' and was introduced to highlight the fact that to maintain homeostasis of function an animal must vary its physiological set points to meet external and internal demands (Sterling & Eyer 1988). AL is the accumulated cost (the 'wear and tear') that results from repeated physiological alterations (e.g. repeated stress responses in a stress-reactive individual), and/or elevated physiological set points or increased physiological 'effort' required to maintain a set point (McEwen & Stellar 1993; Seeman et al. 1997). AL is a useful concept as it provides a conceptual bridge between chronic stress and health outcomes (consequences). In studies of elderly humans, AL is calculated through measurement of physiological activity in various body systems, for example: cortisol, DHEA and catecholamine production and various metabolic variables (Seeman et al. 1997, 2001; Karlamangla et al. 2002). Under increased AL, individuals, although apparently coping with their situation, are more vulnerable to acute events. Increased AL in humans has been associated with a decline in cognitive and physical function, as well as an increased risk of mortality (Seeman et al. 1997, 2001) and may increase the predisposition to depression or other affective disorders (McEwen 2000a). In animal studies, stressor treatments that created an increased allostatic load, using either chronic repeated exposure to acute stressors

(Tannenbaum et al. 2002) or chronic social stress (Quan et al. 2001), caused a decrease in the ability of animals to cope with a standard challenge to the immune system.

2-4-2. Behavioural consequences of chronic stress

2-4-2-1. Overview

The behavioural consequences of chronic stress are generally assessed either by using behavioural tests or by observing abnormalities in behaviour and these are discussed here. More general alterations in behaviour under chronic stress are discussed in section 2-6-2, in relation to depression.

2-4-2-2. Behavioural tests

In this section, two of the most commonly used behavioural tests (the open field test and tonic immobility test) will be discussed. Although these tests are applied in both chickens and pigs (e.g. Gallup & Suarez 1980; Anderson et al. 2000), the open field test will be discussed only with reference to pigs and tonic immobility only with reference to chickens. These tests were carried out in the experimental work described in Chapters Five and Six. Tests such as these are not measures of stress *per se*; rather they provide information about the alterations in factors such as fear levels or exploratory motivations that can occur as a consequence of previous or on-going exposure to stressors.

The open field test is a commonly used measure of emotional responsivity (Archer 1973). In the open field test, the animal is moved to a novel arena larger than its home pen from which it cannot escape. The arena is traditionally left barren and is well illuminated (Jones 1989a). Behaviour seen in the open field is affected by numerous factors, such as social re-instatement motivation, general level of arousal, exploratory motivation and by the animal's level of anxiety or fear (Fraser 1974; Faure et al. 1983; de Passillé et al. 1995; Anderson et al. 2000). These different factors can make open field behaviour, and any changes seen as a result of a particular treatment, difficult to interpret. Although the open field test has most frequently been used as a laboratory 'behavioural assay' for anxiety in rodents (Prut & Belzung 2003), it has also been widely applied to a range of domestic animals: e.g. pigs

(Beilharz & Cox 1967a; Fraser 1974), cattle (Kilgour 1975; de Passillé et al. 1995), poultry (Gallup & Suarez 1980; Jones 1989) and sheep (Kilgour 1998).

In many of the pig studies, the open field test has been used to assess the consistency of response of individual pigs in different situations (e.g. von Borell & Ladewig 1992; Jensen et al. 1995b; Thodberg et al. 1999). It has also been used to assess the effects of various experimental treatments. For instance, Sumner and co-workers (2002) showed that weaning age affected the degree of locomotion in an open field arena. Early (12d old) weaned piglets were more passive than late (42d old) weaned pigs when tested in an open field at 76 days of age. Another study showed that daily unpredictable electric shocks for 33 days caused a decrease in open field exploration and increased locomotion in 20 weeks old pigs (Jensen et al. 1995a).

A complete behavioural inhibition following a brief restraint is seen in a phylogenetically wide range of species. Such inhibition is commonly referred to as tonic immobility. The tonic immobility response involves muscle relaxation causing motor inhibition, altered EEG and nervous activity, increased heart rate and a lack of responses to external stimuli (Jones 1986, 1996; Gentle et al. 1989). These responses are thought to be part of the animal's anti-predator defences (the theory being that predators will not make a killing strike to an immobile body) and the duration that the bird remains immobile is presumed to be positively related to its underlying fearfulness. The duration of immobility is therefore a commonly used measure of fear, particularly in chickens (Jones 1986, 1996). To induce immobility, chickens are firmly restrained on their back by the experimenter. After a short period of time the bird is released and its reaction observed. If immobility is successfully induced the duration until the bird shows a righting response is measured.

Although the duration of immobility is not a direct measure of stress, stress exposure (or conversely, positive experiences) can alter the underlying fearfulness of an animal. For instance, Jones and co-workers (1988) found an increased tonic immobility duration when birds were administered corticosterone. Rodd and co-workers (1997) found that prior exposure to inescapable electric shock caused an increase in immobility duration, compared to exposure to escapable shock or no shock at all. Both early handling and environmental enrichment have been found to decrease the immobility duration of young chicks (Jones & Waddington 1992).

2-4-2-3. 'Abnormal' behaviour

When stress is longer lasting and initial attempts to deal with it have failed, behaviour may be altered in form. It has been suggested that displacement behaviours and stereotypic behaviours are responses to such stress (Maestripieri et al. 1992; Ladewig et al. 1993). However, it is often uncertain whether these forms of behaviour are actually stress responses and whether they are adaptive or not.

Mason (1991ab, 1993) reviewed the concept of stereotypies and pointed out that stereotypies are highly heterogeneous. The implications of this fact are: firstly, that an actual definition of stereotypic behaviour that covers all the different forms may be difficult to produce and, secondly, that any conclusions regarding the relationship between stereotypic behaviour and welfare should be considered as specific to the stereotypy and species involved. It is unlikely that there is a consistent relationship between stereotypy and stress *per se* but there may be a relationship between some forms of stereotypic behaviour and stress in some circumstances. The relationship between stereotypies and physiological indicators of stress, such as HPA activity, is inconclusive (Ladewig et al. 1993). However, physiological indicators of stress may relate to abnormal behaviour during the early stages of stereotypy development but not once the behaviour has become emancipated. What is not in question, however, is that stereotypic behaviours do seem to arise in circumstances that appear aversive.

Chronic stress has also been implicated in the development of various behavioural 'vices' in farm animals. El-lethey and colleagues (2001) have shown that the addition of corticosterone to the diet of laying hens increased the incidence of feather pecking. Similarly, Vestergaard and colleagues (1997) found that feather pecking was correlated with corticosterone concentration in laying hens that were putatively stressed through being deprived of a suitable dustbathing substrate. Although abnormal behaviours such as belly nosing in weaned piglets have been suggested as relating to stress (Dybkjær 1992), Gardner and colleagues (2001) failed to find any evidence that belly nosing was directly related to stress. Tail biting in pigs is also putatively related, at least in part, to stress (Schrøder-Petersen & Simonsen 2001), although there are no studies showing a clear relationship between tail biting and stress indicators.

The fact that various forms of abnormal behaviour may or may not be directly correlated with indicators of stress does not negate their use as indicators of poor welfare. It has been hypothesised that stereotypic behaviour might be a form of stress-coping mechanism (reviewed in Mason 1991). The extent to which the coping hypothesis of stereotypy is accurate is equivocal (Rushen 1993; Cooper & Nicol 1993). However, the validity or otherwise of the coping hypothesis matters less than the fact that the environment has induced the abnormality in the first place.

2-4-3. HPA consequences of chronic stress

Activity in the HPA axis is commonly used in investigations of chronic stress. In some chronic stress states the functioning of the HPA axis can be permanently altered. However, the form of alteration is not consistent; during chronic stress, basal GC concentration can be decreased, increased or unaltered. Decreased GC levels (hypocortisolism) are seen in some patients suffering from post-traumatic stress disorder or chronic fatigue and also in some animal models of chronic stress (Heim et al. 2000). Increased levels are commonly seen in chronic stress, particularly where the normal negative feedback mechanisms are malfunctioning (Habib et al. 2001; Newport & Nemeroff 2001). Where basal levels are unaltered, a stress effect can still be indicated by an exaggerated acute response to a fresh stressor or to an exogenous physiological challenge (e.g. ACTH challenge: Koelkebeck et al. 1986 (chickens); Klemcke 1994 (pigs)). This variation in the potential effects of chronic stress on the HPA axis means that HPA activity may not provide a reliable, or easily interpretable, measure of chronic stress.

One problem with using HPA activity as a measure of long-term welfare in different housing conditions is illustrated by a study that found increased levels in conditions considered good for welfare. De Jong and colleagues (2000b) kept groups of pigs in either enriched or impoverished housing conditions and found that pigs in the enriched conditions had significantly increased glucocorticoid levels during the daylight period. Perhaps the lesson to be learnt from this study is that the best indicators of poor welfare may be deviation from normality, irrespective of the direction of deviation. The problem then becomes identifying what is normal.

2-4-4. Immune consequences of chronic stress

Alterations in immune function are commonly seen as a result of stress. Many of these are caused by GC action (Apanius 1998), although alterations that are independent of GC also occur (Downing & Miyan 2000).

The observation, by Gross and co-workers (1980), that chickens fed a diet containing corticosterone showed an increased number of circulating heterophils and a decrease in the number of lymphocytes, led to the suggestion that the ratio of these cells could provide an indicator of stress in poultry (Gross & Siegel 1983; Maxwell 1993). Since then, many studies have shown the heterophil to lymphocyte (H/L) ratio to be a reliable and useful indicator of chronic stress in poultry (e.g. McFarlane & Curtis 1989). The avian heterophil is equivalent to the mammalian neutrophil and the neutrophil to lymphocyte ratio has also been used as a stress indicator in pigs (Widowski et al. 1989; Puppe et al. 1997).

De Groot and associates (2001) showed that viral immunity is impaired following social stress in pigs, with barrows being more affected than gilts and dominants being more affected than subordinates. This contrasts with studies (Hessing et al. 1994; Tuchscherer et al. 1998) where subordinates were more affected by a challenge. Tuchscherer and co-workers (1998) found that following mixing with unfamiliar pigs, subordinate pigs had a decreased cellular immunocompetance, which they suggest would leave them more susceptible to pathogens.

Contradictions in the literature, with regard to the effect of stress on immune function, are likely to be caused by differences in the nature of the stressor and the measure of immune function used. The immune system is highly complex and a variety of measures are used to assess its function. Some measures, such as mitogen induced lymphocyte proliferation (e.g. Tuchscherer et al. 1998) are carried out *in vitro*. (In this context, a mitogen is any substance that causes inactive lymphocytes to start to undergo cell division: Roitt et al. 1998). Other measures may involve a challenge to the whole animal, either involving an essentially harmless mitogen (e.g. Kelly et al. 2000) or a genuine pathogen challenge (e.g. Hessing et al. 1994). In other cases, on-going measures of immune status, such as various immunoglobulin levels or leucocyte profiles may be measured (e.g. Moore et al. 1994; Tuchscherer et al. 1998). Lastly, the naturally occurring disease incidence can be measured at a group

or herd level. For instance, Simonsen (1995) found that pigs reared in an impoverished environment had three times as many cases of chronic pleuritis than those reared in an enriched environment.

2-4-5. Physical consequences of chronic stress

Over extended periods of stress, biological costs become clearly apparent through various physical indicators. The increased release of GC under stress has many 'downstream' effects, such as decreased muscle mass, arteriosclerosis, osteoporosis, ulceration and dendritic atrophy (von Holst 1998). The activation of a stress response results in a change in the allocation of bodily resources (Siegel & Gross 2000). This can mean that an animal's resources are shifted from growth, reproduction and maintenance to the stress response, resulting in decreased performance. Alterations in these factors can be a useful indication that animals are stressed within a particular environment. However, good production performance is not always associated with good welfare (Murphy 1978; Kelly et al. 2000) and these measures may only be useful as a global measure of a herd, farm or system rather than as an indicator of individual welfare.

In poultry, various egg related parameters can potentially alter under stress. A brief handling stressor decreased egg production in chickens (Hughes & Black 1976). Stress may also cause eggs to be retained in the shell gland, causing an increase in the deposition of calcium on eggs, a phenomenon known as dusting (Carter 1977). Hughes and colleagues (1986) found that a move from pens to cages caused decreased production and increased incidence of surface abnormalities for up to 18 days. Birds housed singly in cages laid fewer eggs with dusting abnormalities than those housed three or four birds to a cage (Mills et al. 1987). Birds that showed more dusting also had longer TI durations, as well as greater avoidance of a novel object and decreased response to handling, indicating that they were more fearful (Mills et al. 1991).

In pigs, the main physical indicator commonly measured is growth rate. When growing pigs were exposed to various stressors (high temperature, increased stocking density and mixing), weight gain was decreased (Hyun et al. 1997). When these individual stressors were combined they had an additive effect, causing a greater retardation of weight gain.

2-5. Potential confounding factors in stress assessment

2-5-1. Overview

There are numerous potential factors that could confound assessments of stress. For instance, there may be stress associated with the taking of a particular sample. Individual variation in stress reactivity is common, and natural variation from ultradian, circadian, seasonal rhythms or from normal alterations in an animal's state exists. These factors are briefly discussed here.

2-5-2. Sampling stress

Perhaps the most basic problem with physiological assessments of stress is that they frequently require an invasive sampling method that can in itself stress the animal. Less invasive techniques such as sampling from hair (Koren et al. 2002), saliva (Cook et al. 1996), urine (Hay et al. 2000), faeces (Kotrschal et al. 1998), milk (Verkerk et al. 1998) or remote blood sampling (Cook et al. 2000) are being developed. However, even these measures may require a degree of interaction with the animal that could alter its responses. It is therefore important to be aware of these potential intrusive effects and, where possible, ensure that they are controlled for in the experimental design.

Although behavioural research is non-invasive and far less intrusive than physiological sampling, it is still the case that care has to be taken to ensure that the observation set up does not affect the functioning of the animal. Many animals view humans as predators (Caine 1992) and the presence of a human observer can affect the behaviour shown by an animal. Although video cameras can be used to avoid this effect, there is still the potential for disturbance to normal behaviour. For instance, Lay and co-workers (1999) found that altering light cycles, to allow overnight video recordings of pig behaviour, affected the behaviour of pigs.

2-5-3. Natural variation: ultradian, circadian and seasonal rhythms

Many of the parameters used in welfare assessment show variation in normal (stress-free) conditions. Physiological variables may show pulsatile release patterns (e.g. Windle et al. 1998), which create ultradian rhythms in their circulating concentration. Many, if not most, biological variables show a distinct diurnal

variation and there may also be longer-term cyclic variation over the seasons of the year (Romero 2002). As with the problems of sampling stress, natural variation in hormone secretion is an inevitable phenomenon in physiological research and experiments have to be designed to deal with this.

Biological parameters within the body can vary in a proactive (predictive) or reactive way to maintain homeostasis (de Kloet et al. 1998; Schulkin 1999). Proactive variation, such as circadian rhythmicity, allows animals to prepare for predictable alterations or challenges in the environment. When assessing stress, the primarily interest is reactive responses. However, the reactive response may vary depending on the phase of the circadian cycle; when hormone levels are naturally high an acute event may cause less of a response than when they are naturally low (Sothern & Roitman-Johnson 2001). In acute stress studies, the fine detail of any secretion pattern may be of less concern if it is carefully controlled for. However, in studies of chronic stress the release pattern may be of primary interest, as an alteration in hormone function may only be identified with a detailed analysis of its release profile (Ladewig et al. 1993).

The physiological state of the animal at the time of challenge may also influence its behavioural response. For instance, rats are more likely to behave aggressively in a resident-intruder set-up if they are on the up phase of their endogenous corticosterone ultradian rhythm, rather than on the down phase (Haller et al. 2000). It is therefore important to be aware that behavioural and physiological function can interact and influence each other bi-directionally. The success of behavioural responses to a stressor can affect the degree of physiological response. Equally, the physiological state of the animal can influence behaviour and perhaps the actual perception of what constitutes a stressor.

2-5-4. Individual variation

One of the most noticeable features of stress responses is individual variation that occurs in addition to the more predictable sources of variation discussed in the previous section. In welfare assessment it is important to understand the nature and cause of such variation. Individual variation can result from stable traits or transient states. Both genetics and early experience contribute to the consistent response pattern of the animal. This can be labelled as responsivity, or more subjectively as

temperament or personality (Boissy 1995; Erhard & Schouten 2001). There may also be more fleeting sources of individual variation – reflecting the current state of the animal – that are commonly viewed as noise in experimental situations but which can be controlled for with careful experimentation.

2-6. Psychological consequences of chronic stress

2-6-1. Depression and animal welfare

Many different forms of adverse psychological consequences may occur in animals. In humans, mental illness[1] can take a wide variety of different forms. Even given the likelihood that animals experience a narrower range of mental experiences than humans, there is still a large amount of potential variation in how poor welfare manifests itself for the animal. One particular form of mental illness that could have relevance for assessments of animal welfare is depression.

Depression is a deleterious mental state that affects a large percentage of the human population at some point in their lives. The principal symptoms of depression are decreased positive affect and increased negative affect (American Psychiatric Association 1994). Although depression is primarily a mental phenomenon, its physical consequences and side effects are becoming clear (Thakore 2001). One of the central pillars of thinking regarding depression is its relationship to stress (Steckler et al. 1999; Blackburn-Munro & Blackburn-Munro 2001). Stressors, both chronic and acute, are considered to be involved in depression, either through increasing the predisposition to become depressed or by directly triggering a bout of depression.

Animal models of depression, commonly involving the application of various stressors, are widely used to test new antidepressant drugs and to help elucidate mechanisms of human depression. Despite this, many authors are unwilling to attribute the ability to suffer from depression to animals. For instance, despite the fact that rodents have been shown to exhibit all the symptoms of depression apart from those requiring verbal self-report (Willner 1997b), Matthews and Reid (1998)

[1] Defined as: "health conditions that are characterized by alterations in thinking, mood, or behavior (or some combination thereof) associated with distress and/or impaired functioning." (U.S.D.H.H.S. 1999).

suggest that "there is no compelling evidence that the rat exhibits, or is even capable of experiencing, any condition that resembles human affective disorder".

Although the concept of depression has received little direct attention in animal welfare studies, the question of whether animals are capable of entering into a depressed state that involves mental suffering is an important one. Many of the stressor treatments used in animal models of depression are qualitatively similar to the stressors that farm animals could experience during their lifetime. Indeed, farm animals may be exposed to stressors that are more severe than those used in current depression models and they may experience chronic-intermittent stress over time periods far greater than would be used in laboratory models of depression.

2-6-2. Behavioural indicators of depression

Although physiological disturbances, such as hypercortisolism, occur in depression the principal indicators of depression are behavioural. Even in human patients, where verbal self-report is possible, diagnosis of depression depends on a behavioural assessment of patients (American Psychiatric Association 1994). Numerous evolutionary theories of depression have been proposed (Price et al. 1994; McGuire & Troisi 1998; Nesse 2000; Sloman & Gilbert 2000; Sloman et al. 2003) in which the behavioural alterations seen during depression are not side effects but are actually the ultimate function of depression. For instance, social theories of depression suggest that it is an adaptive response to failure, preventing animals from challenging dominants when their chances of success are limited (Price 1967; Broom 1998). In this context the feelings of low mood are part of the proximate causation (mechanism) rather than the ultimate causation (function).

The most basic behavioural feature of depression is a generalised behavioural inhibition. Putatively depressed animals show decreases in activity, locomotion, aggression, sexual behaviour and social/affiliative behaviour (Blanchard et al. 2001b). Harlow and Suomi (1974) note the consistent behavioural profile of decreased or 'aimless' locomotion, decreased exploration and deficient social behaviours in many different depression models in monkeys. The list of depressive symptoms identifiable in animals may be extended to include things that were previously thought to be only identifiable through self-report. For instance, Harding and Mendl (2001) attempted to identify if rats behaved in a pessimistic way

following a chronic mild stress treatment. The rats were trained to associate a particular tone with a reward and another tone with lack of reward. When presented with an intermediate tone stressed rats responded less than those from a control group. In a sucrose consumption test the treated rats did not show a decrease in sucrose consumption, so they were not merely less responsive to reward, they were apparently less willing to believe that a reward was likely.

Looking through the literature of stress assessment in pigs it can be seen that stress treatments that could possibly induce depression have been applied to pigs, although none of these studies formally considered the results with depression in mind. Some of these treatments are briefly reviewed here. Most of the studies considered here focus on growing pigs, although it is worth noting that breeding sows may experience many of the same stressors but over an extended time period.

2-6-3. Do stressed pigs exhibit any of the symptoms of depression?

2-6-3-1. Social stressors

Social stress, and in particular, social defeat is being used increasingly commonly as an animal model of depression in laboratory studies (e.g. Koolhaas et al. 1990; Fuchs et al. 1996; Meerlo et al. 1996abc, 1997, 1999; Kudryavtseva & Avgustinovich 1998; Keeney & Hogg 1999; Fuchs & Flügge 2002). Social stress in pigs, caused by mixing unfamiliar animals, has been widely investigated by animal welfare researchers. Following mixing, extensive fighting between pigs is common and causes a clear stress response (Otten et al. 1999; de Jong et al. 2000a; de Groot et al. 2001). In the longer-term the social stress may decrease growth rate (Rundgren & Löfquist 1989; Stookey & Gonyou 1994; D'Eath 2002) and can impair immune responses (de Groot et al. 2001).

Recently, Otten and colleagues (2002) described the results of an experimental mixing. In this experiment, groups of growing pigs were created and the dominant within each group identified. These dominant animals were then removed and housed singly for two to three weeks before being returned to their old groups. In the subsequent interactions only some animals re-gained their dominant status. In the first few hours following the re-grouping the animals that failed to re-gain their dominant status spend less time (relative to those that re-gained their previous

status) exploring the pen and were less active generally, spending more time lying down. In this study the losing animals did show what might be described as a depressed behavioural profile. The behaviour of previously dominant animals differed from previous low-ranking animals in the same situation (Otten et al. 1999), so apparently it is not necessarily the defeat(s) *per se* that caused the behavioural inhibition but the loss of status. It has been suggested that a social defeat for a previously dominant animal may make a useful model of depression in rodents (Willner et al. 1995; but see: Marrow & Brain 1998; Marrow et al. 1999).

The animals in Otten and co-workers (2002) study were only studied over the ten hours after the re-grouping so it is not known if the losers showed any long-term changes in behaviour, physiology or growth rate that might indicate longer-term effects of losing their status. Generally, there are few long-term behavioural studies of mixed pigs. At the time of mixing, animals that lose will show subordinate behaviours and flee from aggressors. The new dominance hierarchy is normally established within the two days following mixing (Meese & Ewbank 1973). However, whether loser pigs show behavioural indicators of depression in the long-term is unknown.

Physiologically, one of the main indicators of depression is hypercortisolism (Newport & Nemeroff 2001). Although an association between high cortisol levels and submissive behaviour in pigs has been suggested (McGlone 1985; Fernandez et al. 1994) the on-going relationship between social status and cortisol levels is variable. De Jonge and co-workers (1996) reared pigs either in an impoverished or an enriched environment. They found that in the impoverished environment subordinate pigs had higher basal levels of cortisol than dominants, whereas when animals were reared in an enriched environment subordinates and dominants had similar levels. Mendl and colleagues (1992) classified sows into high-, low- and no-success groups on the basis of their social interactions. They found that the low-success animals had higher cortisol concentrations and a larger response to an ACTH challenge, compared to the other two groups. So the intermediately successful animals were apparently more stressed than both the highly successful animals and the non-aggressive no-success animals. Tuchscherer and co-workers (1998) measured cortisol in high and low ranking animals, before and three days after an experimental mixing. They found that subordinate pigs had significantly

higher cortisol levels before the mix but that the levels did not statistically differ three days following the mix. Otten and associates (1999) found no difference between high and low ranking animals in their basal cortisol levels.

Some of these inconsistencies could be due to methodological differences. To clarify the situation, what is required is regular measurement of cortisol over long time periods, before, during and after mixing and in stable groups, as well as concomitant behavioural data to allow the social status of individuals to be assessed. One problem may be that within a large group the categorisation into dominant or subordinate is artificially simplistic. There will most likely be a continuum of social status and, as Mendl and colleagues (1992) showed, the stress experienced by any particular animal might not be linearly related to its position in that hierarchy.

2-6-3-2. Isolation

In the maternal separation model of depression (e.g. Harlow & Suomi 1974) infant monkeys were separated from their mothers for periods of many weeks. Following the separation there is an active stage known as the protest stage and then a stage of despair where the infant monkeys showed a decrease in play and exploratory behaviours and a large increase in crying and various abnormal behaviours and postures.

Herskin and Jensen (2000) kept piglets either in complete isolation, in partial isolation or in small groups of littermates for two weeks following weaning. They found that, in an open field test, isolated pigs showed fewer vocalisations, less locomotion and fewer behavioural transitions than partially isolated or socially housed animals. These differences could be explained as a less reactive (passive) response in the isolated pigs. However, they could also be explained by increased reactivity in the other groups. Given their previous lack of experience of isolation it could well be that a larger social re-instatement motivation in these two groups caused an increase in vocalisation and movement around the arena. The groups did also differ in their response to a novel object, with the isolated group spending less time either close to or touching the object. The simplest explanation for this difference is that the isolated pigs were either more fearful or were less interested in exploration.

On the first day of isolation both the fully and partially isolated pigs showed more pawing, more escape attempts and less play behaviour. This response is reminiscent of the despair stage of social isolation shown in primate studies (Harlow & Suomi 1974). Herskin and Jensen (2000) also suggest that the response of the partially isolated pigs was more active that that of the fully isolated pigs. In the primate studies it was similarly found that infant monkeys that were kept in visual contact with their mother had a more severe reaction than those that were completely removed (Harlow & Suomi 1974).

Isolation studies, such as this, can be hard to interpret. For instance, Herskin and Jensen (2000) found a decrease in play behaviour in isolated animals. This is hardly surprising given that other pigs provide the majority of play opportunities for pigs. Like enriched- versus impoverished-environment studies, isolation studies are inherently confounded by the fact that they provide the animal with a different environment to interact with. This leads to the problem that it is hard to identify (using behavioural measures alone) whether any behavioural change is a result of an actual change in the animal or is simply due to the animal interacting with a different environment.

2-6-3-3. Electric shock

One of the best-known models of depression is the learned helplessness model (Overmier & Seligman 1967). In this model, a depressive state is created in animals by the repeated and unpredictable application of a severe stressor, commonly an electric shock. The fact that the animal has no control over the stressor and cannot predict its arrival creates a state of apparent apathy where it effectively stops responding to environmental stimuli. In this state the animal has apparently learnt that its actions have no impact on the occurrence of the stressor and so stops attempting to avoid subsequent stressors.

Mormède and Dantzer (1977) studied the avoidance behaviour of pigs in a set up similar to Overmier and Seligman's. In this set-up, pigs were studied in a room divided in two with a small wooden barrier. The floor on either side of the barrier was made of wire mesh and could be electrified. The imminent occurrence of an electric shock was signalled to the pigs using a tone. On hearing the tone the pigs learnt to avoid a shock by moving to the other side of the barrier. The pigs also

learnt to avoid shocks, without using a signal, when they occurred on a regular pattern. In a further part of the study the authors investigated the effects of exposing some pigs to unavoidable shocks on their subsequent behaviour when shocks could be avoided. They found that pigs that had previously been unavoidably shocked showed a deficiency in their subsequent avoidance behaviour. This is akin to the learned helplessness induced in dogs by Overmier and Seligman (1967) and also seen in other species (see Eisenberg & Carlson 1997).

Jensen and co-workers (1995a, 1996) exposed pigs to 33 days of daily unpredictable exposure to electric shock. This treatment caused a decrease in exploration of an open field but not of a novel object within the open field. It also increased attack latency in a resident-intruder test. The immediate response to the electric shock was found to be more passive near the end of the 33 days than at the start and treated pigs spent more time sitting than controls at this time also.

Olsson and co-workers (1999) exposed pigs that had either been reared in an enriched or an impoverished environment to an electric shock. They found that pigs from an impoverished background showed significantly less avoidance than enriched pigs.

These studies do appear to suggest that pigs may show a form of learned helplessness. The study of Olsson and colleagues (1999) is particularly interesting as it apparently shows an effect induced by poor environmental conditions, rather than by pre-exposure to a severe electric shock. A lack of control may be inherent in intensive housing conditions (Taylor et al. 2001), where animals lack control over access to resources, their social interactions, environmental conditions and in their expression of behavioural needs. Additionally, they may experience uncontrollable and unpredictable stressors. These factors could make states of learned helplessness common in intensive systems.

2-6-3-4. Multiple stressors

A chronic mild stress (CMS) paradigm has been proposed as a model of depression in rodents (Willner 1997bc). In the CMS model, animals are exposed to a series of mild stressors, typically for up to six weeks. The stressors used include: food or water deprivation, altered lighting, cage tilt, group housing, soiled cage, cold room, intermittent white noise, odour or novelty (Willner et al. 1987) and the animals are

always exposed to at least one of these for the whole period. Pigs may similarly be exposed to a combination of various environmental and social insults in normal husbandry conditions.

Dybkjær (1992) kept pigs either with littermates at a low density in a pen with straw (low stressor treatment) or with unfamiliar pigs at twice the density and without straw (high stressor treatment). Pigs in the high stressor treatment spent more time belly nosing, manipulating the ears and tails of other pigs, chewing a chain and sitting passively. This final category is an inactive behaviour, where the pig is seen to look drowsy, and which had previously been suggested to represent an apathetic condition (Ruiterkamp 1987).

An unpleasant handling treatment has been shown to significantly decrease activity in both an enriched and impoverished environment (Pearce et al. 1989). In this experiment, pigs housed in the enriched environment and subjected to unpleasant handling also showed less exploratory behaviour and spent more time resting than those subjected to a pleasant handling experience. This observation is a more convincing indication of a depressed behavioural profile, as the physical environment is constant – so any alteration is due to changes in the animal. However, observations were made around the time of the handling treatment, so it is not known if the difference in behaviour, between pleasantly and unpleasantly handled pigs, persisted beyond the immediate response to avoid the unpleasant handling.

2-6-3-5. Wasting pig syndrome

Smith (1991) has put forward a 'macrophage theory of depression', which suggests that the symptoms of depression in humans are caused by excess cytokine production. Various cytokines (molecular messengers in the immune system) are commonly found to be elevated in depression and this change may be reversed with antidepressant treatment (Connor & Leonard 1998). The increased synthesis of some cytokines during illness results in sickness behaviours (Hart 1988; Dantzer 2001). Dantzer (2001) lists the behavioural effects of exogenous cytokine administration as involving decreases in; general activity, exploratory behaviour, social and sexual behaviour, food and water intake, preference for saccharin, brain self-stimulation and body care activities. Artificially raising cytokine levels creates a behavioural

response that is symptomatically very close to depression (Yirmiya 1996). More recent studies have shown that the effects of exogenous cytokines can be reversed with antidepressant treatment (Shen et al. 1999; Castanon et al. 2001).

As with depression, sickness behaviour is principally associated with behavioural omission, rather than active alterations in behavioural patterns. Fraser and Broom (1997) note that the term 'depression' is widely used in the clinical veterinary literature to describe a state of illness involving "marked reduction in general activity, diminished responsiveness to exteroceptive stimuli and an appearance of reduced awareness in a generalised behavioural atony". Such animals are seen to be passive and most of their activity comes from responses to stimuli directed at them rather than "through spontaneous relationship with the environment".

The wasting syndrome in pigs is a non-specific illness response, characterised by general poor health, listlessness, hair growth, thinning of the skin and eczema and, most prominently, decreased weight gain (Kyriakis 1989; Kyriakis & Andersson 1989). Albinsson and Andersson (1990) proposed that wasting pig syndrome occurs in pigs "unable to cope with the situation following weaning and mixing with unfamiliar pigs". They note the similarities of behaviour between the wasting syndrome and the submissive behaviour of animals under chronic psychosocial stress (e.g. Fuchs et al. 1996; Fuchs & Flügge 2002). Evidence for the role of psychological factors in the wasting pig syndrome comes from the fact that treatment with the antipsychotic drug amperozide ameliorates the syndrome (Kyriakis & Andersson 1989) and can improve weight gain in mixed pigs (Björk 1989). It is not clear, however, whether these beneficial effects are due to an improved coping ability, or alternatively whether they are due to the lowering of aggression within a group (e.g. Barnett et al. 1996), meaning that animals in amperozide treated groups have less stress to deal with.

2-6-4. Influence of 'coping style'

Within a population, variation in how animals respond to different situations is seen. It may be that within a population some animals will be more susceptible to the potentially depressive effects of stressors than others. This is true of the human population and also in animal models of depression.

The issue of whether variation in certain temperament traits might be continuous or bimodal has been strongly debated in ethology. Koolhaas and associates (1999) reviewed the active/passive distinction in coping strategies. In this view, the variation seen in response patterns is not continuous but appears to split animals into either active or passive groupings depending on their behaviour and physiology. The attack latency of mice is one trait that has been proposed to show bimodal variation with some mice showing a short attack-latency (SAL) and another distinct group showing long attack latency (LAL) or not attacking at all (van Oortmerssen et al. 1985; Benus et al. 1991). However, Forkman and co-workers (1995) pointed out that including all non-responders in one category makes the distribution appear bimodal when it may not actually be so. Studies of attack latency in pigs (e.g. Erhard & Mendl 1997; D'Eath & Burn 2002) also found that pigs show a consistent attack latency, meaning that pigs can be classified as SAL or LAL (Erhard et al. 1997; D'Eath 2002). It has recently been shown that LAL mice are more susceptible to chronic social stress in a defeat and sensory contact model than SAL mice (Veenema et al. 2003), so if the same were true of pigs the LAL pigs within a group may be the ones more likely to suffer from the effects of chronic stress.

2-6-5. Summary

None of the studies provide absolute conclusive evidence for a state of depression in the pigs tested. However, it is important to point out that none of them specifically set out to investigate depression in pigs. An experimental assessment of depression might involve observations over longer time periods than normally used. Pigs do, however, show some of the symptoms used in models of depression. Stressor treatments can make pigs less active and exploratory and can elevate cortisol levels. Most studies, such as the mixing experiments, only assess acute responses or responses over a period of, at most, days. An assessment of depression might require observations and measurements over a longer time period. Note, also, that although none of these individual studies provide conclusive evidence, each study generally only considered one potential negative variable. It is entirely possible that in many commercial conditions pigs could be exposed to many if not all (excluding electric shocks and isolation) of the stressors considered in these experiments.

The wasting pig syndrome may actually be the closest parallel to depression in pigs. Behaviourally the syndrome is very close to depression and Johnson (1997) suggested that the wasting syndrome is a result of high levels of pro-inflammatory cytokines. In the medical community there is an increasing view that the behavioural symptoms associated with sickness and with depression may be more than superficially related. The possibility that wasting pigs may, as proposed by Albinsson and Andersson (1990), be suffering from environmental and social challenges beyond their coping capacity deserves further study.

For this, or any other putative state of depression, there is a need for validation with treatments that have been shown to remove depression. In many models, symptoms may be ameliorated by treatments that have antidepressant effects in humans; e.g. antidepressant drugs or sleep-deprivation (Meerlo et al. 1996b). Obviously there is a danger of circularity here: depression is something relived by an antidepressant; antidepressants are substances that relieve depression. However, this approach of behavioural and physiological testing combined with antidepressant treatment, is the standard by which biomedical studies are conducted and is really the only avenue of research available.

2-7. Conclusion

Stress is a complex topic, yet one which is central to the assessment of animal welfare. Welfare assessment involves the measurement of both acute responses and the chronic long-term consequences of lasting stress. All these measurements are made with the aim of assessing in what circumstances animals are likely to suffer. The comprehensive measurement of behavioural, endocrine and immunological variables will provide the best assessment of an animal's welfare state. However, it is important that the basic biology of all these systems is understood before they can be put to use as indicators of welfare (Rushen 2003).

One particular form of mental suffering, depression, was reviewed in more depth. Although the data from pigs are equivocal, the area of depression may represent a useful schema within which welfare can be assessed. The crucial question is whether a depressed behavioural profile involves a concomitant state of suffering. This is of course the fundamental question in animal welfare science. Taking an argument-by-analogy approach it could be decided that (in comparison

to humans) when animals show a broadly similar behavioural profile, in response to broadly similar stressors, they are experiencing a broadly similar phenomenon. Most people have no problem in the application of this principal to physical pain or acute stress. Perhaps the reluctance to attribute the possibility of depression to animals is related to the fact that depression still attracts a lot of stigma in humans. Putting depression in an evolutionary context and emphasising its organic nature has helped to reduce the stigma of depression in human society (Wolpert 2001). Viewing depression as a disease with a physical and physiological basis may mean that its extension to other species is also more readily accepted.

Chapter Three

Fractals and fractal analysis of animal behaviour: a review

3-1. Introduction

This chapter reviews the concept of fractals and describes how analysis of fractal structure has been applied to animal behaviour. Although the application of fractal analysis to behaviour patterns is relatively novel, there have been a number of previous studies that illustrate different possible methodologies and also highlight the sort of information that can be accessed using these methods.

Firstly, the question "what are fractals?" will be posed. Then the concept of fractal analysis will be introduced and the various applications to animal behaviour reviewed. These studies involve fractal analyses of both temporal and spatial data. What the calculated measures actually mean in terms of behaviour is examined. Finally, the potential for fractal analysis to provide a useful measure of stress is briefly discussed.

3-2. What are fractals?

3-2-1. Origins of fractal analysis

The concept of fractals arose from attempts to characterise and measure complex natural phenomena (Mandelbrot 1977). Prior to Mandelbrot's formal definition of fractals, several authors had done work that would later be seen in a fractal context. When Richardson attempted to measure the length of borders between different countries (Richardson 1961) he found that the measured length of a border or a coastline depended on the scale at which the measurement was made. This scaling property is a fundamental feature of fractals and led to Mandelbrot (1967) developing his ideas about fractals. Others, such as Hurst, Korcak (Hurst 1956; Hastings & Sugihara 1993) and Zipf (Zipf 1949), had also identified and analysed power law properties of various data. Since these original studies, structures and processes showing fractal scaling have been found in a wide variety of biological data. Whitfield (2001) even suggests that, given this apparent ubiquity, fractal analysis could provide a 'biological theory of everything'.

3-2-2. Self-similarity and scaling

Fractals are structures that show 'self-similarity' over a range of resolutions. Self-similarity occurs when, after rescaling, small parts of a structure or data set

resemble larger parts or the whole. Such self-similar repetition of structure is common in biology and results in the statistical property of scaling. Scaling means that measured size increases as the measurement units get smaller. Measuring a very complex structure at a smaller scale causes more of the complex fine detail to be revealed and consequently the measured size is larger. For example, the measured length of a complex twisting pathway will be larger (and more accurate) if the measurement scale is changed from metres to centimetres. In fractal structures, the relationship between a property (e.g. length etc) and the resolution (measurement scale) is defined as a power law. A power law relationship between a certain property (P) and a resolution (r) takes the form:

$$P = kr^{f(d)} \quad (1)$$

Where the scaling exponent, f(d), is a function of d, the fractal dimension, and k is a constant (Avnir et al. 1998). Given a set of values of P and r, the exponent can be calculated by plotting P versus r on a log-log scale. A natural log transformation of equation 1 produces a relationship of the form:

$$Ln(P) = Ln(k) + f(d)Ln(r) \quad (2)$$

This equation is of the form of a straight line with intercept Ln(k) and slope f(d). A straight-line relationship on a log-log plot therefore indicates the presence of a power law, with the slope of the line being equal to the scaling exponent. The exponent relates to the degree of scaling and is therefore seen as a measure of complexity. Different exponents indicate alterations in the relationship between the measured property and the measurement resolution. Simple structures show little change in measured value as the measurement scale becomes more precise. However, in complex structures, more precise measurement reveals additional detail and the measured value changes. These differences in the relationship between measurement scale and measured value produce different fractal dimensions.

In idealised fractal geometry, fractal structure occurs at all scales. However, in nature complex structures or processes have fractal properties only over a finite range of scales: typically around one to two orders of magnitude (Avnir et al. 1998).

3-2-3. Sequential and statistical fractals

Fractal analysis methods may be broadly categorised as 'sequential' or 'statistical'. Sequential methods calculate a fractal dimension based on the sequential ordering of the data, while statistical methods assign a dimension to properties of the data that are independent of order; most commonly frequency distributions. Sequential and statistical fractal analysis methods reveal different data properties and both may add extra information to that identified through standard analysis.

3-2-4. Use of fractal analysis

The ability of fractal mathematics to describe complex phenomena means that changes in the organisational structure of the phenomenon in question can be identified. This process can be termed fractal analysis (FA). FA is an umbrella term that embraces many different analysis methodologies based around power law properties in the data and ranging from the relatively simple to the mathematically complex. Peng and associates (2000), note that FA methodologies reveal 'hidden information' which cannot be extracted using conventional analyses.

FA has been used in a wide range of fields. For example, in seismology it has been applied to the temporal pattern of earthquakes and their magnitude and in meteorology it has been applied to rainfall patterns and temperature fluctuations (Hastings & Sugihara 1993; Govindan et al. 2001; Christensen et al. 2002). In ecology, fractal measures can be used to describe the diversity of species in a community as well as other parameters (Frontier 1987; Sugihara & May 1990; Johnson et al. 1995). In economics, fractal methods have been used to investigate structure within stock market fluctuations (Grau-Carles 2001). FA methods have also been applied to such diverse subjects as the immune system (Burgos & Moreno-Tovar 1996), the structure of the universe (Borgani 1995) and linguistics (Vilensky 1996).

However, since Mandelbrot's initial exposition of the fractals concept one of the most common applications of FA has been in human medical physiology (Bassingthwaighte et al. 1994; Goldberger et al. 2002a). In this field, FA is

increasingly being seen as being practically useful for diagnostic purposes. A common finding in many studies is that temporal patterns (such as heart rate fluctuations) become more regular and predictable with age or disease (Goldberger 1997). FA of heart rate variability can differentiate between patients on the basis of previous heart conditions (Saermark et al. 2000) and may prove useful as a predictor of future risk of heart problems (Ho et al. 1997).

3-2-5. Animal behaviour studies

FA has been applied to animal behaviour data in a variety of different ways. Although the original fractal methods were developed for geometric analysis, when applied to animal behaviour FA can be used to measure complexity either temporally or spatially.

The importance of assessing temporal organisation in addition to standard statistics is illustrated in Figure 3-1. Three data series were created to have exactly the same summary statistics, yet show markedly different temporal organisation. The standard approach in behavioural studies might be to analyse the total time spent in a particular behaviour over an observation period – in this case the three series would be found to be identical. Even a cursory look at the graph shows that the series are far from identical, yet this sort of information is often overlooked in behavioural studies.

Studies that have applied FA to behaviour patterns are summarised in Table 3-1, in chronological order. Inclusion of studies in the table was based on a loose definition of both what counted as a FA and what counted as a behaviour (e.g. studies of human psychiatric disorder are included). This was a deliberate policy to provide as comprehensive a list as possible. Repetitions of the same method by the same authors are included under the references in any one line. Conversely, where different methods are used in the same study each is recorded as a different entry.

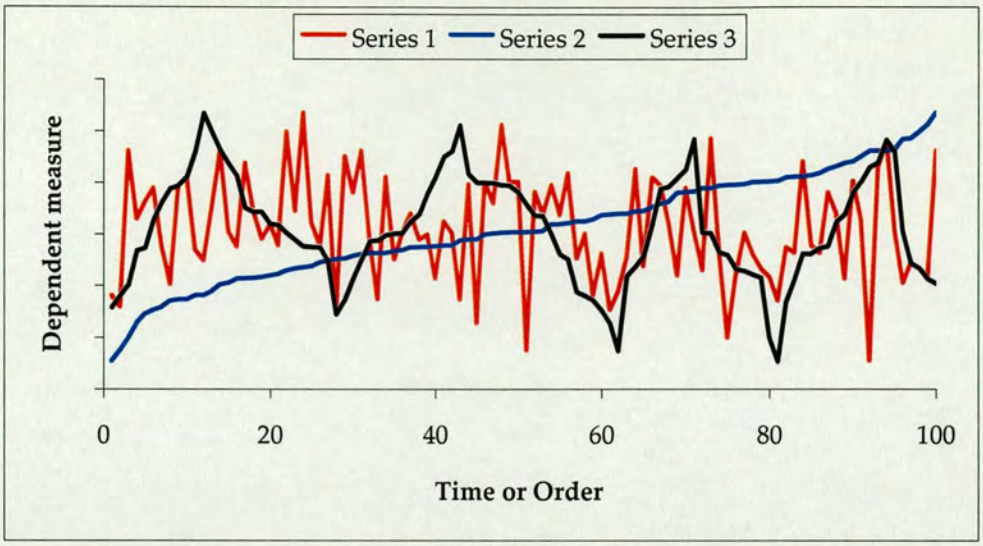


Figure 3-1: Simulated data to illustrate the importance of temporal organisation

These three data series are statistically identical in terms of their total, mean, standard deviation and distribution. However, their temporal organisation differs substantially. For instance, imagine that the dependent variable is the duration of 100 individual instances of a particular behaviour over time. Series 1 implies that the underlying behavioural control is entirely random; there is no sequence to the series. In series 2, each duration is larger than the one before; the underlying process is altering over time. Finally, in series 3 the underlying process waxes and wanes over time.

Table 3-1: Summary of studies that have applied fractal analysis or power laws to behavioural patterns

		Method			
Species	**Behaviour**	**Temporal/ Spatial**	**Statistical/ Sequential**	**Details**	**Reference**
Human	Writing, various others	Temporal	Statistical	Log-log plot (LLP) of frequency versus rank for various parameters	Zipf 1949
Mites	Movement	Spatial	Statistical	LLP of path length versus measurement resolution	Dicke & Burrough 1988
Rat	Movement events	Spatial	Statistical	LLP of path length versus measurement resolution	Paulus & Geyer 1991
Rat	Movement	Temporal	Statistical	LLP of frequency versus duration	Paulus & Geyer 1991
Pink-clownfish larvae	Swimming	Spatial	Statistical	LLP of step length versus number of steps	Coughlin et al. 1992
Beetle	Movement	Spatial	Statistical	LLP of path length versus measurement resolution	Crist et al. 1992
Ant	Search Behaviour	Spatial	Statistical	LLP of path length versus measurement resolution	Fourcassie et al. 1992
Sheep	Movement	Spatial	Statistical	LLP of home range size versus number of location observations	Gautestad & Mysterud 1993
Rat	Activity	Temporal	Sequential	Spectral analysis	Motohashi et al. 1993
Drosophila	Feeding times	Temporal	Statistical	LLP of cumulative frequency versus duration	Shimada et al. 1993, 1995
Drosophila	Inactivity	Temporal	Statistical	LLP of frequency versus duration	Cole 1995

Table 3-1 continued

		Method			
Species	**Behaviour**	**Temporal/ Spatial**	**Statistical/ Sequential**	**Details**	**Reference**
Limpet	Movement	Spatial	Statistical	LLP of path length versus measurement resolution	Erlandsson & Kostylev 1995
Human	Cognition	Temporal	Sequential	Spectral analysis	Gilden et al. 1995
Human	Mood disorder	Temporal	Sequential	Spectral analysis	Gottschalk et al. 1995
Beetle, Ant, Grasshopper	Movement	Spatial	Statistical	LLP of path length versus measurement resolution	Wiens et al. 1995
Ibex	Feeding gaps	Temporal	Statistical	LLP of cumulative frequency versus duration	Alados et al. 1996
Ibex	Vigilance/ feeding	Temporal	Sequential	Spectral analysis	Alados et al. 1996
Ibex	Vigilance	Temporal	Sequential	LLP of cumulative frequency of head lifting versus time interval	Alados et al. 1996
Human	Schizophrenic symptoms	Temporal	Sequential	Spectral analysis	Dunki & Ambuhl 1996; Dunki et al. 2000
Human	Gait	Temporal	Sequential	Detrended Fluctuation Analysis (DFA)	Hausdorff et al. 1995, 1996, 1997

Table 3-1 continued

		Method			
Species	**Behaviour**	**Temporal/ Spatial**	**Statistical/ Sequential**	**Details**	**Reference**
Rat	Movement	Temporal	Sequential	Spectral analysis	Kafetzopoulos et al. 1997
Vole, Deer Mouse, Marten	Movement	Spatial	Statistical	LLP of path length versus measurement resolution	Nams 1996, submitted; Nams & Bourgeois submitted
Placozoan				Paper not reviewed	Ueda et al. 1996
Albatross	Foraging flights	Temporal	Statistical	LLP of frequency versus duration	Viswanathan et al. 1996
Albatross	Foraging flights	Temporal	Sequential	DFA	Viswanathan et al. 1996
Human	Eye movements	Temporal	Statistical	LLP of cumulative frequency versus duration	Yokoyama et al. 1996
Wolf	Movement	Spatial		Paper not reviewed	Bascompte & Vila 1997
Soil amoebae	Movement	Spatial	Statistical	LLP of mean displacement versus time	Levandowsky et al. 1997
Polar bear	Movement	Spatial	Statistical	"Line segment method", no further details	Ferguson et al. 1998
Caribou	Movement	Spatial		Paper not reviewed	Ferguson et al. 1998

Table 3-1 continued

		Method			
Species	**Behaviour**	**Temporal/ Spatial**	**Statistical/ Sequential**	**Details**	**Reference**
Goldfish				Paper not reviewed	Nepomnyashchikh 1998
Human	Tourette tics	Temporal	Statistical	LLP of frequency versus duration	Peterson & Leckman 1998
Human	Tourette tics	Temporal	Sequential	Spectral analysis	Peterson & Leckman 1998
Minnow	Reproductive	Temporal	Sequential	DFA	Alados & Weber 1999
Human	Activity	Temporal	Statistical	LLP of average second moment of a activity counting process versus counting time	Bickel 1999, 2000
Soil nematodes	Movement	Spatial	Statistical	LLP of path length versus measurement resolution	Kampichler 1999
Ant	Food carrying	Temporal	Statistical	LLP of frequency versus rank	Kitabayashi et al. 1999
Drosophila	Locomotor	Temporal	Statistical	LLP of frequency versus duration	Martin et al. 1999, 2001
Dolphin	Whistle	Repertoire	Statistical	LLP of frequency versus rank	McCowan et al. 1999
Human	Mood fluctuations	Temporal	Sequential	Spectral analysis	Paulus et al. 1999b
Bumble bee	Foraging flights	Temporal	Statistical	LLP of frequency versus duration	Viswanathan et al. 1999

Table 3-1 continued

		Method			
Species	**Behaviour**	**Temporal/ Spatial**	**Statistical/ Sequential**	**Details**	**Reference**
Deer	Foraging	Temporal	Statistical	LLP of frequency versus duration	Viswanathan et al. 1999
Human	Binary choice test	Temporal	Sequential	LL plot of mutual information decay against time intervals	Woyshville et al. 1999
Chimpanzee	Social	Temporal	Sequential	DFA	Alados & Huffman 2000
Pig	Nesting	Temporal	Sequential	LLP of behavioural sequences versus their probability	Harnos et al. 2000
Mouse	Feeding	Temporal	Sequential	Spectral analysis	Kurokawa et al. 2000
Flycatcher	Movement	Spatial	Statistical	Nams (1996) method	Westcott & Graham 2000
Birds	Song	Repertoire	Statistical	LLP of Number of songs versus number of syllable types	Changizi 2001
Human	Swaying	Temporal	Sequential	DFA	Duarte & Zatsiorsky 2001
Human	Swaying	Temporal	Sequential	Spectral analysis	Duarte & Zatsiorsky 2001
Stoat	Movement	Spatial	Statistical	Nams (1996) method	Edwards et al. 2001

Table 3-1 continued

		Method			
Species	**Behaviour**	**Temporal/ Spatial**	**Statistical/ Sequential**	**Details**	**Reference**
Fin whale	Movement	Spatial		Paper not reviewed	Mouillot & Viale 2001
Copepod	Movement	Spatial		Paper not reviewed	Schmitt & Seuront 2001
Jackal	Movement	Temporal	Sequential	DFA	Atkinson et al. 2002
Jackal	Movement	Spatial	Statistical	LLP of path length versus measurement resolution	Atkinson et al. 2002
Mice	Operant response	Temporal		Paper not reviewed	Li & Huston 2002
Reindeer	Foraging	Spatial	Statistical	LLP of frequency of movement lengths	Mårell et al. 2002
Chicken grunt	Movement	Spatial		Paper not reviewed	Suzuki et al. 2003
Pigeon	Operant response	Temporal		Paper not reviewed	Killeen 2003
Beetle	Movement	Spatial	Statistical	LLP of path length versus measurement resolution	Bangert & Slobodchikoff In Press

3-3. Temporal fractal analysis of behaviour

3-3-1. Statistical methods

Alados and co-workers (1996) applied a statistical fractal analysis method to the feeding behaviour of free-ranging Spanish ibex. They plotted the cumulative frequency of interval durations on a log-log plot. In this case, the property of interest is the cumulative frequency and the resolution is the size of interval. The fractal dimension calculated using this measure was lower in parasitised Ibex compared to controls. This decrease in fractal dimension of feeding gaps represents an increased number of large gaps and a decreased number of small gaps. Despite this change parasitised animals spent just as long feeding overall as the other animals.

Viswanathan and associates (1996) found that a power law related the frequency and duration of flights shown by Albatrosses. That is, when the frequency distribution of flight durations was plotted on a double log scale, frequency and duration were linearly related. They describe this relationship as a lévy flight, which are "a special class of random walks whose step lengths are not constant but rather are chosen from a probability distribution with a power-law tail". This means that there are very many short durations and a few infrequent large durations.

Fractal structure has also been found in the temporal pattern of activity and feeding in drosophila (Shimada et al. 1993, 1995; Cole 1995; Martin et al. 1999). Cole (1995) found that a power law related the frequency and duration of inactivity. Given this fractal pattern, estimations of the amount of time a particular fly is inactive depend on the measurement resolution used, i.e. at finer resolutions flies appear more inactive (Cole 1995). In fractal terms inactivity has no absolute characteristic scale. Similarly, Martin and co-workers (1999) found that a power law described the cumulative frequency distribution of inactive intervals. They identified differences in the scaling exponent between laboratory strains and between the sexes and also found that the complexity of behaviour was age dependent, with the pattern increasing in regularity with increasing age. Martin and co-workers (2001) note "that the fractal structure is a robust intrinsic parameter characterizing the temporal pattern, since it is independent of activity level".

3-3-2. Sequential analysis - Detrended Fluctuation Analysis

Alados and co-workers have used a technique called Detrended Fluctuation Analysis (DFA) to analyse behaviour patterns (Alados & Weber 1999; Alados & Huffman 2000). This method was introduced by Peng and colleagues (1995a) to analyse DNA sequences. The first application to behavioural data was by Viswanathan and co-workers in 1996. The DFA method will be applied in subsequent experimental work (Chapters Four, Five and Six).

When applied to behaviour, DFA measures the complexity of fluctuation between two behavioural states. In this case complexity refers to the randomness of the pattern of fluctuation back and forth between the two states. Fluctuations of higher complexity are those in which the switching is closer to a random pattern. Randomness is measured through an assessment of the long-range autocorrelation within the data series. Long-range autocorrelation indicates that the value of a particular variable depends not only on the immediately preceding values but also on values much earlier in the sequence (i.e. the sequence has 'memory'). As the long-range autocorrelation of the data increases the patterns become more structured and less complex. When the autocorrelation within the sequence decays quicker (i.e. there is less long-range autocorrelation) the series is more randomly structured. The value (α) calculated by DFA can be used to interpret the structure of the data series. If α equals 0.5, the series is said to be uncorrelated (random), while if it is greater than 0.5 the series is said to show long-range autocorrelation in the form of persistence (Peng et al. 1995b, 2000) This means that on-going behaviour is influenced by what has occurred in the past and that states are more likely to persist than change. If the α value is less than 0.5 the sequence is said to show anti-persistence (i.e. the process is more likely to change than stay in the current state). Note that the DFA α exponent is inversely related to a typical fractal dimension, so in this case the value increases with increasing regularity (decreasing complexity) in the time series. DFA has the advantage of removing the effects of non-stationarity in the data, which could give the false appearance of self-similarity, by fitting a series of best-fit lines (Hausdorff et al. 1996; Peng et al. 2000). Methodological descriptions and investigations of DFA have been provided by a number of authors (Hausdorff et al. 1996; Heneghan & McDarby 2000; Peng et al. 2000; Hu et al. 2001; Kantelhardt et al. 2001; Vjushin et al. 2001; Chen et al. 2002; Willson et al. 2002).

Alados and Weber (1999) applied DFA to the reproductive behavioural patterns of male Fathead minnows that had been exposed to lead in their water, either before or after they reached sexual maturity. They found that reproductive behaviours such as hovering or patrolling and the time spent under the breeding substrate all had higher α values in males exposed to lead before sexual maturity but not in those exposed after. So there was an age-dependent effect, with lead causing more auto-correlated behavioural patterns in animals exposed to lead early in life. However, there was also a large effect of early lead exposure on standard measures of behavioural frequency in these same behavioural categories: hovering frequency was decreased by 84.2%, patrolling frequency was increased by 241.6% and time under the breeding substrate was increased by 79.5%[1].

Alados and Huffman (2000) also applied DFA to chimpanzee behaviour. They studied the complexity of fluctuations between social and non-social behaviours in relation to the health status (as indicated by examination of stool samples and behavioural indicators of sickness) of both male and female wild chimpanzees. Females were found to have a more complex structure to their social behaviour than males. This complexity was reduced in sick females whereas health status did not affect behavioural complexity in males. However, this comparison is based on a comparison of two healthy females versus seven sick females and this cannot be seen as a meaningful statistical comparison. There is also a degree of pseudoreplication as repeated observations from individuals are used.

Hausdorff and colleagues have applied DFA to human gait patterns. They found that the sequence of stride intervals during normal walking shows long-range autocorrelation (Hausdorff et al. 1995). They note that although a cursory examination of stride interval might conclude that it was approximately constant, given that its coefficient of variation is only 4%, DFA shows that what little variation there is, cannot be explained as simply being the result of random noise. The stride interval pattern becomes more random in elderly people and in people suffering from Huntington's disease (Hausdorff et al. 1997), whereas mean stride interval does not differ between these groups. Within the group of Huntington's suffers, the α value was related to the degree of disease impairment such that those with the

[1] These values were calculated from the data given in Table 1 of Alados and Weber 1999

most severe impairment had the lowest α values (the most random sequences of stride intervals).

3-3-3. Sequential analysis - Spectral analysis

Spectral analysis is a relatively common analysis method that breaks down data series into numerous different sine waves of different wavelength (Chatfield 1980; Forrest & Suter 1994). In mathematical terms, spectral analysis is used to estimate the 'spectral density function' (or 'spectrum') of a data series. The spectral density function describes how the power of a series is distributed across different frequencies (Chatfield 1980). The power of each wavelength is calculated as the square of the amplitude. In some cases power and frequency may be related by a power law, e.g. when power, P(f) is plotted against the frequency, f, of each component wave on a double log plot the result is a straight line. In fractal terms, referring back to equation one, the power is the property and the frequency is the resolution.

$$P(f)=kf^{\alpha} \qquad (3)$$

The form of the relationship describes the contribution that different frequencies make to the series as a whole (Gisiger 2001). The power law relationship means that low frequency waves have high amplitude and vice versa (Ehlers 1995). Variations in the α value indicate alteration in the balance between frequency and amplitude, e.g. there may be a relative increase of high frequency components compared to low frequency components or vice versa. A lower α indicates a series with relatively higher amplitude high frequency components, while an increased α value indicates a shift in the balance towards low frequency components, i.e. a less complex pattern.

Alados and colleagues (1996) also applied spectral analysis to sequences of feeding and vigilance behaviour in ibex. They found a significant difference between parasitised and healthy ibex, such that the parasitised animals had a less complex behavioural pattern (higher α exponent).

Various authors have applied spectral analysis to rodent behaviour (Motohashi et al. 1993; Kafetzopoulos et al. 1997; Kurokawa et al. 2000). The most germane of these studies is by Motohashi and co-workers (1993) who applied spectral analysis

to the locomotor activity of rats and assessed the effects of administering a toxic solvent. They found that the fractal organisation of the rats' locomotor behaviour was significantly affected in a dose-dependent way by intra-peritoneal injection of the solvent. In treated animals the exponent value was increased, indicating a decreased behavioural complexity.

A small number of studies have applied spectral analysis to time series of human psychiatric disorders. Dunki and Ambuhl (1996) applied spectral analysis to the time series of daily reports of schizophrenic intensity and found a power law relationship between spectral power and frequency. Similarly, Gottschalk and colleagues (1995) and Woyshville and colleagues (1999) analysed the sequence of daily self-assessments of mood in patients suffering from a mood disorder and in controls, using spectral analysis. The results of these studies are consistent in that both found that the α value for patients with a mood disorder was significantly greater than that for controls. So although the patients show significantly lower and more variable mood scores than the controls, their sequence of mood variation was less complex.

3-4. Spatial fractal analysis of behaviour

In a series of studies, Paulus and Geyer, and various colleagues, have applied a spatial FA to the movements of laboratory rodents. They calculate a spatial scaling exponent from movement patterns within a small arena (Paulus & Geyer 1991, 1993). The analysis is based on the alteration in estimates of the distance travelled at different measurement resolutions. In this assessment an exponent of one would equal an animal moving in a straight line (when the measurement of total distance travelled is constant at every resolution), while increases in the exponent above one indicate an increasing degree of complexity in the movement pattern (Fig. 3-2). This measure of pattern complexity is independent of total locomotor activity (Paulus et al. 1999a). When the method was applied to the behaviour patterns of different rat strains, Paulus and co-workers (1998b) found that the different strains had significantly different movement patterns, but the same total level of activity. Thus, the pattern and amount of locomotor activity provide different dimensions for describing behaviour. Using this analysis, the authors have studied the behavioural effects of various environmental or drug treatments. They show, for instance, that

rats reared in isolation following weaning had a less complex movement pattern than socially reared rats (Paulus et al. 1998a). When two rat strains that differ in stress susceptibility were tested following isolation or social rearing the more stress susceptible strain showed less of a difference between the two treatments (Paulus et al. 2000). This appears to suggest that the stressful effects of the novel arena oppose the effects of the isolation.

It has also been shown that mice lacking the gene for a dopamine transporter (rendering them hyperdopaminergic) show significantly less complex movement patterns (Ralph et al. 2001b; Ralph-Williams et al. 2003). This lowered complexity (in their terms 'perseverative behaviour') was partially ameliorated by treatment with valproate, a drug used to treat mania in bipolar disorder (Ralph-Williams 2003). Administration of amphetamine also decreased the complexity of behaviour (Ralph et al. 2001a).

In their original study, Paulus and Geyer (1991) also calculated a temporal scaling exponent. They then plotted the effects of various drugs on a two dimensional graph, with the two dimensions being the temporal and spatial exponents. This plot showed that different drugs could have different effects on the two dimensions. Some drugs principally affected behaviour in only one of the dimensions, e.g. spatial structure changed whilst temporal structure remained constant (amphetamine) or vice versa (nicotine). Other drugs caused behaviour to change simultaneously in both dimensions (apomorphine).

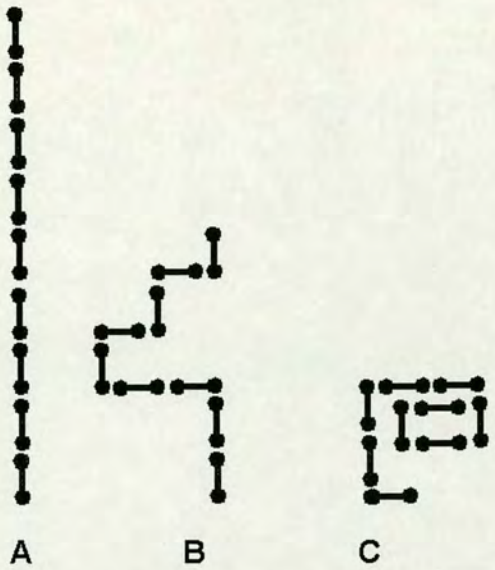


Figure 3-2: Stylised movement patterns of differing complexity (after Varty et al. 2000)

Each path involves nine units of movement, but with differing scaling exponents (d) in each case. A: d = 1, B: d ≈ 1.5, C: d = 2.

3-5. Interpretation of fractal complexity in animal behaviour

3-5-1. Definitions of complexity

The fractal dimension calculated through FA is deemed to be a measure of complexity in a structure or process. However, although 'complexity' is a fairly everyday term it is often unclear what exactly it refers to. There are many different conceptions of what is meant by 'complexity', so it is important to be clear when discussing complexity what version is meant.

The simplest definition of complexity is simple heterogeneity or diversity. However, although heterogeneity and diversity are both relevant measures of behaviour, they do not relate to the sorts of complexity measured by fractal analyses. The two most common definitions of complexity are polar opposites (de Wailly 1998). On the one hand, complexity can be ascribed to systems with a high degree of organization; for example, a jet plane is a highly complex flying machine. Conversely, complexity may be attributed to entirely unpredictable and chaotic systems e.g. the movement pattern of a small helium balloon floating freely in the

sky would be almost totally unpredictable. Since, in this view, the maximum value of complexity is complete randomness (de Wailly 1998), a jet plane would be described as relatively simple as its every function should be predictable and regimented and the movement of a balloon would be highly complex. It is this version of complexity that is relevant for the studies using DFA.

3-5-2. Why does behaviour commonly have a fractal structure?

"Why?" questions, such as the one posed here are common in biology. Traditionally, there are two separate ways of answering these questions: proximate and ultimate. Proximate answers to the question of why something occurs are concerned with the mechanisms that generate the particular phenomenon. In behavioural terms, proximate answers to questions about causation might invoke changes in hormones or responses to external stimuli. For instance, feeding behaviour may be caused by changes in the levels of substances such as leptin or it could be socially facilitated by seeing conspecifics eating. Ultimate answers refer to the evolutionary causation of a particular behaviour. In the case of feeding, the evolutionary causation of the behaviour is clear: animals that do not eat die. However, with other behavioural patterns it may be harder to identify candidate adaptive function.

It appears that in many cases behaviour patterns do show a fractal organisation. This leads to the question of whether this structure is functional or merely an organisational epiphenomenon. The behavioural output of an animal is a reflection of the complex organization of different motivational priorities, determined by both internal and external stimuli (e.g. Toates 2000). In some cases, behaviour may appear entirely random, while in other instances behavioural patterns and events may be extremely predictable. These extremes may have been selected for over evolutionary history. Alternatively, some patterns of behaviour may be epiphenomena that have not been under any selective pressure.

In the case of prey vigilance it has been hypothesised that chaotic unpredictable (complex) behaviour patterns will be adaptive (Pulliam 1973; Elgar & Catterall 1981). Alados and colleagues (1996) suggest:

> " A prey species, faced with constraints...must maximise predator detection by using its allotted vigilance time in such a way as to fill the time most effectively in its feeding sequences: that means frequent and unpredictable

> (complex) head lifting patterns, in short, a high dimensional pattern of head-lifts"

It has also been suggested that, in certain instances, fractal movement patterns may make efficient search patterns. Cole (1995) produced a simple model that suggests that a fractal search pattern was more efficient than a random search pattern. Similarly, Viswanathan and associates (1996) suggest that a fractal pattern could provide an efficient search pattern for foraging, by causing the animal to visit more new sites and decrease the chances of re-visiting an old foraging site. Viswanathan and colleagues (2000, 2001, 2002) therefore argue that the lévy distribution of flight durations represents an efficient foraging pattern. Atkinson and co-workers (2002) suggest:

> "Where individuals compete for resources, there may be selection pressure in favour of Lévy flights and against normal random (Brownian) movements, because Lévy flights are quicker to find new areas to exploit".

Alternatively, the complexity in flight patterns could simply arise from complexity in the distribution of food or other environmental resources (Viswanathan et al. 1996). Fractal movement patterns are commonly explained as relating to fractal properties of the environment. Gautestad and Mysterud (1993) note that the fractal scaling of home range size they found in sheep could be the result of fractal structure in the landscape (e.g. Burrough 1981; Sugihara & May 1990; Johnson et al. 1995). Bangert and Slobodchikoff (In Press) found that darkling beetles had a more complex fractal movement pattern in areas containing ground squirrels than in areas free of ground squirrels. They explained this as being due to the effect that the squirrels had on local landscape structure – the digging behaviour of the squirrels created a more complex landscape for the beetles. In this case the complexity of behaviour is a direct result of the landscape complexity.

On a more general level, it may be that a degree of complexity in behaviour patterns is beneficial in dealing with events in the environment. In physiological studies, complexity/irregularity is considered healthy as it allows the system to readily adapt to meet challenges (Pool 1989; Goldberger 1997). Might a certain complexity in behaviour be adaptive: keeping the animal 'on its toes' to deal with complex challenging environment? Complexity allows different ways of responding and different ways of interacting with the surrounding environment. Various authors (Pool 1989; Lipsitz & Goldberger 1992; Goldberger et al. 1997, 2002ab) have

hypothesised that with age or disease comes a loss of complexity in biological functioning, which results in a decreased ability to cope with challenges. Alados and Weber (1999) state:

> "a predictable behavior is one that repeats activity in a regular pattern; however, its information content is poor. As a result, it is less adaptable and less able to cope with the exigencies of an unpredictable changing environment"

It is of course possible that the complexity seen in behavioural sequences is not functional. Fractal structure could merely be an emergent property of the complex interplay between different motivational states. For example, internet usage patterns follow a power law, as do the patterns of forest fires (Carlson & Doyle 2002), without there being any guiding principal. Many authors point out the ubiquity of fractal or power law structure in the output of complex systems (e.g. Changizi 2001; Gisiger 2001; Whitfield 2001; Brown et al. 2002). It is possible that a fractal organisation is simply the result of self-organisation rather than the result of any selection force.

It appears most likely that each of the above possibilities could be true for different behaviours and different species. There may well be a function in some instances of fractal organisation in behaviour patterns. The models presented by Viswanathan and his colleagues (Viswanathan et al. 1996, 2000, 2001, 2002) and by Cole (1995) do provide some evidence for fractal search patterns being superior to other possible ways of organising behaviour. The extension of this theory to vigilance also seems reasonable. In either case, the optimal behavioural pattern may vary depending on environmental conditions. Any functional benefit of fractal behavioural patterns or more generally behavioural complexity, in terms of coping with environmental challenge is still an untested hypothesis. The fact that behavioural complexity can change under energetically costly conditions does not itself mean that high behavioural complexity has a beneficial function. Finally, some fractal behavioural patterns may well occur as nothing more than epiphenomena – the output of a complex system with many competing elements.

3-6. Fractal analysis of behaviour as an indicator of stress

As noted previously, when a property of a system can be measured it then becomes possible to identify when that property might change. Various examples of FA

applied to behaviour show that the method allows hidden information about organisational properties to be identified (e.g. Alados et al. 1996; Martin et al. 1999; Paulus et al. 1999). Fractal parameters can vary between individuals in which other behavioural parameters remain constant (e.g. Alados et al. 1996; Paulus et al. 1998) and conversely fractal parameters can remain constant while other parameters vary (e.g. Crist et al. 1992). In these examples the other parameters relate to quantities while the fractal statistic relates to qualitative features of form or pattern. These features are rarely considered and it is this fact that makes FA a potentially exciting analysis methodology.

As discussed previously, Alados and various co-workers have investigated the potential of FA as an indicator of well being in animals. Alados and co-workers (1996) found that pregnancy or parasitism altered the fractal pattern of vigilance and feeding behaviours in Spanish ibex. Both pregnant and parasitised animals were found to have a less complex behaviour pattern compared to control animals. What is interesting about this study is that standard behavioural measures did not differ between pregnant or parasitised animals and controls. So, pregnant animals spent as long feeding and showed as many head-lifts as non-pregnant animals, yet the temporal pattern of these behaviours was significantly altered.

As discussed previously, recently studies showing a reduction in complexity in the reproductive behaviour of Fathead minnows exposed to lead (Alados & Weber 1999) and in the social behaviour of diseased chimpanzees (Alados & Huffman 2000) have been published. Motohashi and colleagues (1993) showed that rat locomotor behaviour became less complex following administration of a toxic solvent. Alados et al. (1996) note:

> "Because stress, whether in the form of genetic inbreeding, pregnancy, disease, presence of toxic substances or social disharmony, increases metabolic rate and, in consequence, energy consumption, it follows that it will generally lead to a reduction [in behavioural complexity] even when the human eye sees little or no change in behaviour".

The common thread connecting these studies is the repeated result that when some form of stress places a load or cost (i.e. allostatic load; McEwen 2000a) upon an animal the behavioural pattern of that animal becomes increasingly regular and predictable. This is similar to many of the human physiology studies, which have found increasing regularity in age and disease (e.g. Goldberger 1997). Alados proposes that this decrease in behavioural complexity is caused by increased

metabolic rate and energy consumption caused by stress (Alados et al. 1996). In the case of pregnancy or parasitism it is quite likely that an animal's metabolism is altered. Pregnancy is obviously costly and parasitism is costly both through direct effects of the parasites and potentially also because of a cost of any immune response (Moret & Schmid-Hempel 2000; Read & Allen 2000). However, it is unclear how this theory might extend to different forms of stress, particularly those encountered by animals in intensive agricultural systems. The 'stressors' in Alados's studies are very different in form to the stressors that are considered problematic from a welfare perspective. The situations analysed by Alados were not necessarily related to animal suffering as such, so whatever is true for these situations may not be true for other forms of stress, different stressors, or for stress on different time scales.

However, overall, the literature provides plenty of evidence that fractal analysis can produce novel measures of behavioural organisation that alter in various circumstances, some involving a decrease in the animals' well-being and some not. These studies certainly suggest that fractal analysis of behaviour could be a potentially useful measure of a particular aspect of animal functioning. This leads to the necessity that fractal measures be investigated in the context of welfare assessment.

3-7. Discussion

3-7-1. Fractal analysis is a potentially useful behavioural analysis methodology

FA is an extensively used analysis method for describing and measuring aspects of complexity in a wide variety of different areas. Examples from a number of fields show that FA can identify hidden information about organisational properties of complex systems. In many cases this hidden information allows an assessment of when those properties change or differ in different situations. One benefit of FA is that it allows a single parameter to be calculated that meaningfully describes global aspects of behavioural complexity. Paulus & Geyer (1991) note:

> " The fact that the occurrence frequency of these micro-event durations is described simply by a function that can be characterised by a scaling exponent allows an enormous contraction of information into one number".

However, this fact has also been described as a potential problem. Simberloff and colleagues (1987) suggest that any description of a complex system that is reduced down to one value, in this case the fractal dimension, runs the risk of subsuming "so much information into one number that the result will be uninterpretable or uninteresting". This criticism is valid, as it would indeed be wrong to use the fractal dimension as the only piece of information describing a system. It is better to view it as an additional piece of information to be used along with other ways of describing behavioural sequences.

Numerous studies have applied FA to animal behaviour patterns. These studies have involved many different methods across a variety of species and behaviours (Table 3-1). They show that fractal scaling is commonly found to exist in behavioural patterns. (It is tempting to say that fractal scaling is ubiquitous in behaviour but there may well be a publication bias, which means that studies where FA was applied unsuccessfully never appear in print). Various treatments or differing situations have been shown to alter the fractal dimension of behaviour. These come under various categories: genetic, environmental, physiological, pharmacological, neural etc. Fractal structure of behaviour has been show to be affected by sex (Martin et al. 1999), strain (Paulus et al. 1998; Martin et al. 1999), age (Coughlin et al. 1992; Martin et al. 1999), environment (Shimada et al. 1993, 1995), rearing conditions (Paulus et al. 1998) and health status (Alados et al. 1996; Alados & Huffman 2000). Various externally applied physiological treatments have also altered fractal structure of behaviour (Paulus & Geyer 1991; Motohashi et al. 1993; Alados & Weber 1999). Spatial fractal measures have been shown to alter in animals with increased extra-cellular dopamine (Ralph et al. 2001a; Ralph-Williams et al. 2003). Martin and associates (2002) show that specific collections of neurons are responsible for the fractal structure of activity in Drosophila. In studies of psychiatric disorders, FA identifies differences between schizophrenic or depressed patients and controls in various aspects of behaviour. Gottschalk and co-workers (1995) propose that the degree of 'mood organization', as measured by the fractal properties of the power spectrum, could reflect the severity of bipolar disorder in patients.

Alados in a series of papers, with her various collaborators (Alados et al. 1996; Alados & Weber 1999; Alados & Huffman 2000), has suggested that, when applied

to animal behaviour patterns, FA could reveal information about an animal's state of well-being. The series of studies carried out by Geyer and Paulus, along with various colleagues (Paulus & Geyer 1991; Paulus et al. 1998, 1999; Ralph-Williams 2003), also suggests that FA of behaviour is a useful diagnostic tool. This group have proceeded to the stage where their FA of behaviour is used as an experimental tool (e.g. Ralph-Williams et al. 2003).

Paulus and Braff (2003) note the value of obtaining a measure of the 'temporal architecture' of behavioural sequences and the additional information this can reveal, beyond that given by traditional statistical measures. They state that:

> "carefully characterizing the temporal domain of the biological system at hand can lead to important insights into the function and dysfunction of the underlying biological substrates. In this conceptual framework, average measures...are important, but the sequence and timing of events yields distinct and crucial information for understanding these relationships".

This is a good description of the potential benefits that a fractal analysis could bring to behavioural analysis. Although it remains to be determined if FA will prove useful in welfare assessment, there can be no doubt that it can reveal interesting and novel parameters relating to behavioural organisation.

3-7-2. Some things still need to be established

At the current time FA is becoming less of a novel method. If any of the available fractal measures are to prove useful in animal welfare assessment a more detailed inquiry into them will be necessary. A failing with Alados' studies (Alados et al. 1996; Alados & Weber 1999; Alados & Huffman 2000) is that no validating measures were taken in order to relate alteration in behavioural function to alteration in welfare state. If a FA method is to prove useful as a tool in welfare assessment then it is necessary that it be properly validated against currently available indices of poor welfare. Also, the degree to which the analysis really does reveal hidden information (i.e. information beyond that revealed during standard analyses) must be established. The interest in FA that led to this project being established came from Alados and colleagues' first published study (Alados et al. 1996) where fractal measures of behaviour were seen to alter when standard measures did not. However, subsequent work has either found that standard measures of behavioural frequency or duration were grossly altered at the same time as changes in fractal

organisation were seen (Alados & Weber 1999), or has not given any evidence to indicate either way (Alados & Huffman 2000). When standard measures of behaviour change, it is questionable whether a FA provides any extra useful information. Certainly it needs to be established whether fractal measures, always, never, or sometimes relate to standard measures, i.e. whether an alteration in quantity directly leads to an alteration in pattern. McSharry and colleagues (2003) recently challenged a previous medical application of a non-linear analysis (Martinerie et al. 1998) on this basis stating:

> "Ultimately, the operational use of proposed complicated statistics can be justified only by showing that they out-perform well-understood traditional statistics...or provide complementary information".

Finally, it is worth noting that there are some reasons that suggest caution when interpreting Alados's work. The experiment outlined in Alados et al. (1996) was also described in another paper (Escos et al. 1995). This led to an editorial rebuke from the editor of the journal Oikos (Malmer 1997), who expressed concern over the fact that the results were different in the two papers despite the same test being applied on the same data.

3-8. Conclusions

Fentress (1976) notes: "One approach to the problem of generalisation is to seek dimensions of behavioural organisation that can be applied to different specific activities". FA could provide just such a dimension, allowing general statements about the organisation of different behavioural patterns to be made.

The use of FA of behaviour provides a tool for the 'dynamical phenotyping' (Goldberger et al. 2002b) of behaviour. It remains to be seen if the information provided by FA will indeed prove useful in animal welfare assessment. However, at this time it is still possible to say that the ability to extract more information from the data we gather can never be detrimental. Calculation of a fractal exponent, as a description of statistical or sequential properties of behavioural organisation, provides additional information above and beyond that provided by simpler analyses of mean frequencies and durations of behaviour. It may be that FA will allow the identification of fundamental qualities of normal behaviour and provide a tool for quantifying deviation from normality.

Chapter Four

Detrended fluctuation analysis of behavioural responses to mild acute stressors in domestic hens

Abstract

Fractal analysis provides a novel measure of behavioural complexity and has previously revealed subtle alterations in behaviour under biologically costly conditions, such as parasitism or disease. The analysis is based upon the temporal pattern of behaviour that, although rarely considered in behavioural studies, may provide information in addition to standard measures of duration and frequency. Such information could be useful in assessing the welfare of confined animals.

The hypothesis that fractal analysis reveals novel behavioural alterations during stress was tested using ISA Brown pullets. The behaviour of undisturbed birds in their home pen was compared to the behaviour of the same birds: 1) in a novel arena, 2) in their home pen following blood withdrawal and 3) in their home pen following five minutes of mechanical restraint plus blood withdrawal. Detrended Fluctuation Analysis (DFA), which calculates fractal complexity measures for time series data, was applied to sequences of vigilance behaviour and activity. These two behavioural parameters where chosen because they are relatively simple to measure and might be expected to alter under stress.

When compared to home pen behaviour, complexity in vigilance behaviour increased in the novel arena ($P < 0.001$) and following restraint and blood sampling ($P < 0.05$) but was unaltered following blood withdrawal only ($P = 0.36$). Total time spent vigilant was increased in the novel arena ($P = 0.001$) but not following restraint ($P = 0.45$) or blood withdrawal ($P = 0.11$). The complexity of activity patterns and the total time spent active were similar in all situations.

In conclusion, DFA provides a novel measure of temporal behavioural complexity in chickens. In contrast to studies of chronic situations in other animals, acute stress caused an increase in behavioural complexity in the present experiment. This increased complexity occurred independently of changes in the duration of behaviour suggesting that DFA can reveal more subtle changes in behavioural organisation during stress. If such behavioural alteration represents a non-specific stress response this methodology could allow objective comparisons of different stressors to be made.

4-1. Introduction

Behavioural analysis has an important role in the assessment of stress in animals. A concern though is that behavioural responses may be highly specific to individual stressors, particularly in the case of acute stressors (Dawkins 1999; Rushen 2000). This may limit comparison of different stressors because it is not possible to reliably compare the severity of selected stressors if they elicit responses that vary qualitatively rather than quantitatively (Rushen 2000). Therefore, in order to judge the relative severity of different events or situations it is necessary to be able to measure stress effects on a single non-specific scale. One parameter that might potentially meet these requirements is behavioural complexity. Recently, fractal analysis has emerged as a potentially useful measure of behavioural complexity.

The original concept of fractals arose from attempts to mathematically characterise complex patterns in nature (Mandelbrot 1977). A key feature of fractal patterns is the statistical property of scaling. In this context scaling means that the properties of the structure or process vary with the scale or resolution of analysis. For instance, in geometry, measuring a very complex object at a smaller scale means more of the complex fine detail is revealed and the measured size is larger. For a fractal object or process, a power law describes the relationship between measured size and measurement scale. In fractal analysis the degree of scaling is measured and assigned a parameter, typically called the fractal dimension, which is seen as a measure of complexity. Since fractals can be used to describe complex systems they can therefore also identify when the properties of those systems change. For instance, fractal analysis of heart rate variability can differentiate between patients on the basis of previous heart conditions (Saermark et al. 2000) and may prove useful as a predictor of future risk of heart problems (Ho et al. 1997).

Fractal analysis of animal behaviour has been proposed as an indicator of well being in various species (Alados et al. 1996; Alados & Weber 1999; Alados & Huffman 2000). Pregnant or parasitised Spanish ibex were found to have a less complex pattern of vigilance and feeding behaviours than controls (Alados et al. 1996). Interestingly, despite the fact that the temporal pattern of these behaviours was significantly altered standard behavioural measures did not differ, e.g. pregnant animals spent as long feeding and showed as many head-lifts as non-pregnant animals. More recently, lowered complexity in the reproductive behaviour

of fathead minnows exposed to lead (Alados & Weber 1999) and in the social behaviour of diseased chimpanzees (Alados & Huffman 2000) has been reported. Thus fractal analysis may reveal 'hidden information' (*sensu* Peng et al. 2000) about the organisation of behaviour beyond that extracted using conventional behavioural analyses, which are often limited to measures of mean duration or frequency of particular behaviours. These later studies (Alados & Weber 1999; Alados & Huffman 2000) use a form of fractal analysis called Detrended Fluctuation Analysis (DFA), which is also applied here.

The present experiment investigated whether a fractal analysis technique could be used to identify general properties of behavioural complexity and if these measures might alter in mildly stressful conditions. DFA was applied to the behaviour patterns of chickens that remained undisturbed in their home pen, or that were exposed to the mild stress of blood sampling, mechanical restraint plus blood sampling or placement in a novel arena. Mechanical restraint using a crush cage is a standard experimental stressor in poultry. For example, restraint in a crush cage for five minutes elevated plasma corticosterone concentration in Japanese quail (Jones et al. 1994) and such a response was used as a criterion for selective breeding of high and low stress lines of quail (Satterlee & Johnson 1988). Manual restraint also elevated plasma corticosterone in chickens (Korte et al. 1997; van Hierden et al. 2002) and the adrenocortical response may begin as soon as 45 seconds after immobilisation (Beuving & Vonder 1978). Restraint also caused a significant increase in plasma adrenaline and noradrenaline levels (Beuving & Blokhuis 1997). Exposing animals to an unfamiliar environment (usually referred to as an open field) is a commonly used test of fear and anxiety. Both the novelty value and lack of shelter within the novel arena are likely to cause fear, which is a potent stressor (Jones 1996). The blood sampling and restraint procedures used here also involve handling and transient social isolation, both of which are likely to be stressful.

The pattern of vigilance behaviour, as crudely measured by head lifting, was identified as of potential use. Vigilance behaviour tends to be embedded within whatever other behaviours the animal is motivated to perform. For this reason it provides a relatively constant stream of events that can be simplified into the binary on/off (vigilant/non-vigilant) pattern necessary for DFA. It was also thought likely that vigilance could potentially alter under stress (Rushen 2000). The other

behavioural fluctuation pattern chosen for assessment was that between activity and inactivity.

4-2. Animals, materials and methods

4-2-1. Animals and housing

Forty-eight ISA Brown hens were reared to 11 weeks of age in cages and then transferred to floor pens (105x100cm) bedded with wood shavings, one week before the first observation. The birds were housed in pairs in the cages and these pairs were maintained in the floor pens. The mean weight of these birds was 1.18kg. One bird from each pair was randomly designated as the test bird, with the other acting as a companion. The birds were identified by leg rings. After transfer to floor pens all birds were food deprived for five hours each day (either from 08.00 to 13.00, 10.30 to 15.30 or 12.00 to 17.00, to tie in with experimental treatments). The food deprivation period was used to increase motivation to feed, to ensure a period of active behaviour during the subsequent observations. Water was always available. Birds were kept on a 14L:10D light regime, with lights on from 08.00 to 22.00h. On test days, when behaviour was recorded, 100g of pelleted food was scattered into the pen after 4 hours of deprivation, at which point the observation began.

4-2-2. Test situations

Observations (details below) were made in the following test situations.

Home pen. Three repeated observations (HP1, HP2 & HP3) of the behaviour of birds that remained undisturbed in their home pen were made.

Novel arena (NA). The test and companion birds were transferred to a novel test arena (Fig. 4-1). This involved carrying them in a wire holding cage approximately 100m down a corridor to another room. When both birds had been placed in the test arena, food was scattered and the observation began.

Blood sampling (BS). The test bird was removed from the home pen to an adjacent room and restrained manually while 2ml of blood was removed from the brachial vein. The bird was then returned to the home pen, food was scattered within the pen and the observation began.

Restraint (R). The test bird was removed from the home pen and restrained for five minutes in a mechanical crush cage (Fig. 4-2). The crush cage was in the same room as the other birds but the test bird was physically and visually isolated from its companion. After this five-minute period, the bird was removed from the cage and a 2ml blood-sample was taken. The bird was then returned to the home pen, food was scattered and the observation began.

4-2-3. Observation details

The bird's behaviour was recorded onto videotape in each test situation, using a camera located either at the front of their home pen or above the novel arena. During the observation period the experimenter did not enter the room. A simple ethogram (Table 4-1) consisting of events and mutually exclusive states was used to classify the test birds' behaviour. The timing of behavioural events and transitions between states was determined from the video recordings using the Keytime computer program (Deag, 1993). The observation length was 3072 seconds (51 minutes and 12 seconds) to fit in with the DFA (see section 4-2-4). Each bird was observed a total of six times. The birds were divided into two batches that differed only in the order they received the BS or R treatments. In the first batch, the observation order was: HP1, NA, BS, HP2, R, HP3. In the second batch, the observation order was: HP1, NA, R, HP2, BS, HP3. There was an interval of three or four days between each observation on the same bird. Each set of observations took three days (eight birds were observed each day; three at 12.00, three at 14.30 and two at 16.00). Each bird was observed at the same time of day in each test situation.

Some observations were not recorded onto computer because of major disturbances during recording that were beyond the experimenter's control. There were also some instances when the companion obscured the test bird for lengthy periods and these were also discarded. The resulting samples sizes were therefore: n=24 for the home pen observations, n=23 for the novel arena observation, n=16 for the observations following the blood sampling only treatment and n=17 for the observations following the blood sampling and restraint treatment.

Table 4-1: Ethogram definitions, based on references in the literature[1]

	Definition
States	
Stand head up	Bird is upright with sternum clear off ground, stationary on one or both legs. Head is above horizontal midline of body, back of head higher than line of back.
Stand head down	Bird is upright with sternum clear off ground, stationary on one or both legs. Head is below horizontal midline of body, back of head below line of back.
Stand preen	Bird is upright with sternum clear off ground, stationary on one or both legs. Bird directs attention with beak (pecking, stroking, combing or nibbling) towards body and feathers.
Walk head up	Bird takes at least two consecutive steps with head up.
Walk head down	Bird takes at least two consecutive steps with head down.
Sit head up	Bird's body resting on ground, with head up.
Sit head down	Bird's body resting on ground, with head down.
Sit preen	Bird's body resting on ground, preening.
Dustbathe	Bird engages in dustbathing behaviour, kicking litter onto body and wiggling body about in dust. Feathers ruffled.
Events	
Wing flap	Quick repeated movement of wings. Bird may or may not be moving.
Wing stretch	Either bilateral or unilateral, upward and sideways extension of wing(s).
Test peck	Test birds directs peck towards companion.
Companion peck	Companion directs peck towards test bird.
Drink	Bird pecks at water drinker.
Body shake	Feathers raised and body shakes.
Head shake	Head is moved rapidly from side-to-side.
Ground scratch	Bird scratches in sawdust making backward strokes with leg: typically body moves down and bird moves forward then back.

[1]**Kruijt 1964; Black & Hughes 1974; Wood-Gush 1989**

Figure 4-1: Novel arena
Three novel arenas were used in different soundproof rooms (floor dimensions, cm: 120x120 (two arenas), or 140x140) with three pairs of birds being tested simultaneously. The walls of the arena were made of unpainted plywood, wood shavings were spread on the floor and a wire mesh roof prevented escape.

Figure 4-2: Crush cage
The crush cage consisted of a box made of stainless steel mesh with a moveable, solid, internal partition. The bird was placed in one end of the cage and the partition was moved up against the bird until it was unable to turn around. This degree of restraint allowed the bird some forward-backward movement and did not impair respiration.

4-2-4. Detrended Fluctuation Analysis (DFA)

The method used was based on previous behavioural work (Alados & Weber 1999, Alados & Huffman 2000) and is also described by Peng and associates (2000). Here, only the method for analysing the fluctuation between vigilance and non-vigilance will be described but the same process was also applied to the active/inactive fluctuation pattern (where activity was defined as walking with the head up or down). The behavioural data were recorded in the form of a time series of events and mutually exclusive behavioural/postural states. Events and the times they occurred were discarded from this record leaving a series of times representing changes between the mutually exclusive behavioural states. For DFA purposes these were combined into binary states, i.e. standing head-up, sitting head-up and walking head-up are all classified as vigilant while all the other behaviours are non-vigilant. Behaviour was then classified as vigilant or non-vigilant at half-second time points (equation 1).

$$xi = \begin{cases} 1 \text{ if bird is vigilant} \\ -1 \text{ if bird is otherwise} \end{cases} \quad (1)$$

A 'Cumulative Vigilance Score' (y) was then created by adding one to the variable at each time point (i) if the behaviour was vigilant and subtracting one if it was non-vigilant (equation 2) (see Fig. 4-3a for an example).

$$yi = \sum_{j=1}^{i} xj \quad (2)$$

The observation length was set at 3072 seconds (51 minutes and 12 seconds). At a resolution of half a second this yielded a time series of 6144 data. The continuous time series (y_i) was subdivided up into m non-overlapping 'windows', within each of which a regression line was fitted (equation 3). The value of m followed the sequence: 2^2, $2^{2.5}$, 2^3, $2^{3.5}$.... 2^{10} (rounded to the nearest integer; 4, 6, 8, 11, ...1024). The size of each window was represented by n and decreased from 1536 at m=4 down to only six (representing three seconds of behaviour) at m=1024. Since n was not necessarily always an integer value the regression lines were fitted into windows according to equation 4 below, where k, rounded down to the nearest integer, represented the particular window number (1 to m). The degree of fluctuation (F: the root mean square of the errors), at varying values of n, was then calculated (equation 5).

$$\hat{y}_i^{(n)} = a_{k(i,n)} + b_{k(i,n)}i \tag{3}$$

$$k(i,n) = \left[\frac{i-1}{n}\right] + 1 \tag{4}$$

$$F(n) = \sqrt{\frac{1}{6144}\sum_{i=1}^{6144}(yi - \hat{y}_i^{(n)})^2} \tag{5}$$

Once the calculation of F at different window sizes was complete, window size was plotted against fluctuation value on a log-log scale (equation 6) (see Fig. 4-3b for an example). Typically the fluctuation value was much larger at large window sizes. As window size decreases the regression lines become more closely fitted to the data and the measure of fluctuation decreases. A straight line in the log-log plot indicates that a power law relates window size and fluctuation, with the slope of the log-log plot equal to the power law exponent, α (equation 7).

$$Ln[F(n)] = Ln(a) + \alpha Ln(n) \tag{6}$$

$$F(n) = an^{\alpha} \tag{7}$$

The α value relates to the auto-correlation structure of the time series. In this case if it equals 0.5 the series is said to be uncorrelated (random), while if it is greater than 0.5 the series is said to show long-range autocorrelation. This means that on-going behaviour is influenced by what has occurred in the past. Note that in DFA the α exponent is inversely related to a typical fractal dimension, so the value increases with increasing regularity (decreasing complexity) in the time series.

4-2-5. Statistical Analysis

Total behavioural duration (for each state) or frequency (for events) was calculated for each observation in Keytime. For each parameter (either behavioural durations or frequencies, or DFA exponents) a single home pen value (HP: the mean of all three repeated home pen observations) was calculated for each bird. This was then subtracted from the novel arena (NA), blood sample (BS) or the restraint plus blood sample (R) values (i.e. each bird was used as its own control). One-sample t-tests were used to determine if the resulting value differed from zero. The α values for vigilance and activity calculated using DFA are referred to as Vα and Aα.

4-3. Results

4-3-1. Behaviour - Standard measures of duration and frequency

Within the home pen, on average 47.8% of the observation period was spent in vigilant postures/behaviours (see Table 4-2 for the descriptive statistics for vigilance and activity and Table 4-3 for values for the individual behavioural states). In the novel arena there was a significant increase in the total time spent vigilant, principally due to an increase in standing with head up and to decreased standing preening, sitting preening and sitting with head-down. There was no detectable effect of the blood sampling or the restraint and blood sampling procedures on vigilance when the bird was returned to the home pen. The average occurrence of vigilant states in the home pen was not significantly altered in the novel arena or following blood sampling, although there was a trend towards a slight increase following restraint. The mean bout length of vigilance was significantly increased in the novel arena but not following blood sampling or restraint plus blood sampling. The total duration of vigilance in the home pen was negatively correlated with the change in vigilance in the novel arena ($r= 0.66$ $P<0.01$), such that birds with a low level of home pen vigilance showed a larger change than those with a high level of home pen vigilance.

There was no alteration in the total time spent active or the number of bouts of activity in the novel arena, or following blood sampling or restraint plus blood sampling compared to the home pen observations. There was a small yet significant increase in the mean duration of activity in the novel arena, but no change following blood sampling or restraint plus blood sampling compared to the undistributed home pen observations.

Scratching, drinking and head shaking were the most common events in the home pen (Table 4-4). In the novel arena the frequencies of stretching, test pecking and scratching were all reduced. Following blood sampling stretching was also reduced and there were trends towards decreased flapping and scratching. Following the restraint plus blood sampling procedure there were reductions in stretching and drinking and trends towards decreased flapping and scratching and increased headshaking.

Table 4-2: Descriptive statistics (means + standard errors) for vigilance and activity recorded during the home pen observations (HP), and the mean differences between those values and those recorded in the other three treatments: novel arena (NA), blood sampling (BS) and restraint plus blood sampling (R).

	Behavioural category					
	Vigilance			Activity		
	Duration (seconds)	Total number of bouts	Mean length (seconds)	Duration (seconds)	Total number of bouts	Mean length (seconds)
HP n=24	1469.3 (54.1)	154.9 (4.9)	10 (0.65)	128.9 (14.3)	75.3 (6.8)	1.6 (0.04)
NA-HP:	416 (111.1)	-4.46 (8.3)	2.76 (1.3)	45.2 (32.8)	9 (11.2)	0.32 (0.1)
n=23	t= 3.74 P=0.001	t=-0.54 P=0.6	t=2.11 P=0.046	t=1.38 P=0.18	t=0.8 P=0.43	t=3.21 P=0.004
BS-HP:	153 (90)	3.55 (5.3)	0.41 (0.77)	9.2 (28)	4.8 (13.6)	-0.05 (0.06)
n=16	t=1.7 P=0.11	t=0.67 P=0.51	t=0.54 P=0.6	t=0.33 P=0.75	t=0.35 P=0.73	t=-0.83 P=0.42
R-HP:	94.5 (121.6)	16.32 (9.1)	-0.36 (1.8)	-22.7 (22)	-10 (13.4)	-0.14 (0.09)
n=17	t=0.78 P=0.45	t=1.79 P=0.09	t=-0.2 P=0.85	t=-1.03 P=0.32	t=-0.75 P=0.46	t=-1.51 P=0.15

Table 4-3: The durations (seconds: means + standard errors) of behavioural states recorded during the home pen observations (HP), and the mean differences between those values and those recorded in the other three treatments: novel arena (NA), blood sampling (BS) and restraint plus blood sampling (R)

	Behavioural State								
	Stand-head up	Stand-head down	Stand-preen	Walk-head up	Walk-head down	Sit-head up	Sit-head down	Sit-preen	Dustbathe
HP n=24	1024.5 (53.1)	984.8 (71.3)	220.8 (29)	96.5 (13.9)	32.45 (5.21)	289.5 (35.4)	129 (22.5)	235.6 (34.8)	58.9 (27.5)
NA-HP:	340.4 (89.9)	63.4 (120.2)	-176.5 (32.9)	21.4 (31.8)	23.8 (9.3)	113.8 (127.4)	-108.7 (23.9)	-218 (37.8)	-59.6 (28.7)
n=23	t=3.79, P=0.001	t=0.53, P=0.6	t=-5.37, P<0.001	t=0.67, P=0.51	t=2.56, P=0.018	t=0.89, P=0.38	t=-4.56, P=0.002	t=-5.76, P<0.001	t=-2.08, P=0.05
BS-HP:	76.3 (85.1)	-244.6 (128.1)	-27.9 (41.8)	9.1 (24.9)	0.1 (5.5)	127.5 (79.6)	38.3 (53.3)	73.5 (56)	-59.9 (39.9)
n=16	t=0.90, P=0.38	t=-1.91, P=0.075	t=-0.67, P=0.51	t=0.36, P=0.72	t=0.02, P=0.98	t=1.6, P=0.13	t=0.72, P=0.48	t=1.31, P=0.21	t=-1.5, P=0.15
R-HP:	78.6 (115.7)	-237.1 (120.5)	121.1 (64)	-11.6 (20.1)	-11.1 (7.4)	42.8 (93.5)	34.1 (47)	-5 (44.1)	-15.3 (56.7)
n=17	t=0.68, P=0.51	t=-1.97, P=0.067	t=1.89, P=0.077	t=-0.58, P=0.57	t=-1.49, P=0.16	t=0.46, P=0.65	t=0.73, P=0.48	t=-0.11, P=0.91	t=-0.27, P=0.79

Table 4-4: The frequencies (means + standard errors) of behavioural events recorded during the home pen observations (HP), and the mean differences between those values and those recorded in the other three treatments: novel arena (NA), blood sampling (BS) and restraint plus blood sampling (R).

	Behavioural Event							
	Wing Flap	Wing Stretch	Test peck	Companion peck	Drink	Body Shake	Head Shake	Ground Scratch
HP n=24	1.8 (0.26)	2.7 (0.29)	7.6 (2.46)	2.6 (1)	24 (5.35)	1.3 (0.17)	11 (2.12)	27.3 (5.1)
NA-HP:	-0.23 (0.4)	-1.4 (0.46)	-5.93 (2.59)	1.65 (1.55)		0.5 (0.3)	-4.56 (2.72)	-21.08 (4.99)
n=23	t=-0.58 P=0.57	t=-3.04 P< 0.01	t=-2.29 P=0.03	t=1.06 P=0.3		t=1.66 P=0.11	t=1.68 P=0.11	t=-4.22 P<0.001
BS-HP:	-0.83 (0.43)	-1.83 (0.48)	-1.55 (2.42)	-2.15 (1.67)	-5.58 (4.94)	0.15 (0.28)	5.85 (4.85)	-12.64 (6.75)
n=16	t=-1.94 P=0.07	t=-3.84 P<0.01	t=-0.64 P=0.53	t=-1.28 P=0.22	t=-1.13 P=0.28	t=0.52 P=0.61	t=1.21 P=0.25	t=-1.87 P=0.08
R-HP:	-0.61 (0.32)	-1.98 (0.47)	-2.05 (3.98)	2.61 (3.12)	-15.48 (5.85)	0.24 (0.42)	9.98 (5.77)	-8.43 (4.28)
n=17	t=-1.89 P=0.08	t=-4.2 P<0.001	t=-0.51 P=0.61	t=0.84 P=0.41	t=-2.65 P=0.02	t=0.56 P=0.59	t=1.73 P=0.10	t=-1.97 P=0.07

Note: drinking was not possible in the novel arena.

4-3-2. Behaviour - Detrended Fluctuation Analysis

The original time series for the smallest and largest $V\alpha$ (over all observations) are plotted (Fig. 4-3a) to illustrate extremes of complexity and regularity. Despite the large difference in the fractal structure of their vigilance behaviour, these two birds showed almost exactly the same total amount of vigilance over the observation period: the bird with the highest $V\alpha$ (indicating low behavioural complexity) spent 24min, 25s vigilant, while the bird with the lowest $V\alpha$ (indicating the most random vigilance pattern) spent 24min, 26s vigilant. Figure 4-3b shows the double-log plots of fluctuation against window size for these two series. For all the regressions on the log-log plots the mean t value was 56.18 (St. Dev. = 15.01). The t value at a significance level of P=0.001 would be 2.947 and all the regression lines exceeded this value thus indicating high goodness of fit.

The mean home pen $V\alpha$ of 0.98 (S.D. = 0.042, range= 0.90, 1.08) was significantly reduced (indicating increased complexity) in the novel arena (NA- HP, mean = -0.04, t=-4.44, df=22, P<0.001) and following restraint (R-HP, mean = -0.02, t=-2.42, df=16, P<0.05) but it was not significantly altered following blood sampling (BS-HP, mean = -0.009, t= -0.94, df=15, P=0.36). There was no effect of the order of blood sampling and restraint plus blood sampling, nor was there an interaction between treatment and order (GLM: Order effect, $F_{1,32}$=0.00, P=0.99; Interaction, $F_{1,32}$ =0.98, P=0.33).

The DFA on activity pattern produced a mean home pen $A\alpha$ of 0.70 (S.D. = 0.051, range = 0.58, 0.81). This did not alter significantly in the novel arena (NA-HP, mean= -0.01, t=-0.66, df=22, P=0.52), or after blood sampling (BS-HP, mean = 0.004, t=0.28, df=15, P=0.79) or restraint plus blood sampling (R-HP, mean = 0.004, t=0.38, df=16, P=0.71).

$V\alpha$ did not correlate with the total duration of vigilance shown (home pen observations only: r=0.16, n=24, P=0.45, all observations: r=0.086, n=80, P=0.45). This suggests that the complexity of the vigilance pattern is not simply a function of the total duration of vigilance shown. However, for activity there was a strong correlation between $A\alpha$ and the total duration of activity (home pen observations: r=0.75, n=24, P<0.001, all observations: r=0.55, n=80, P<0.001).

Further analysis and description of these data is presented in section 7-2-2.

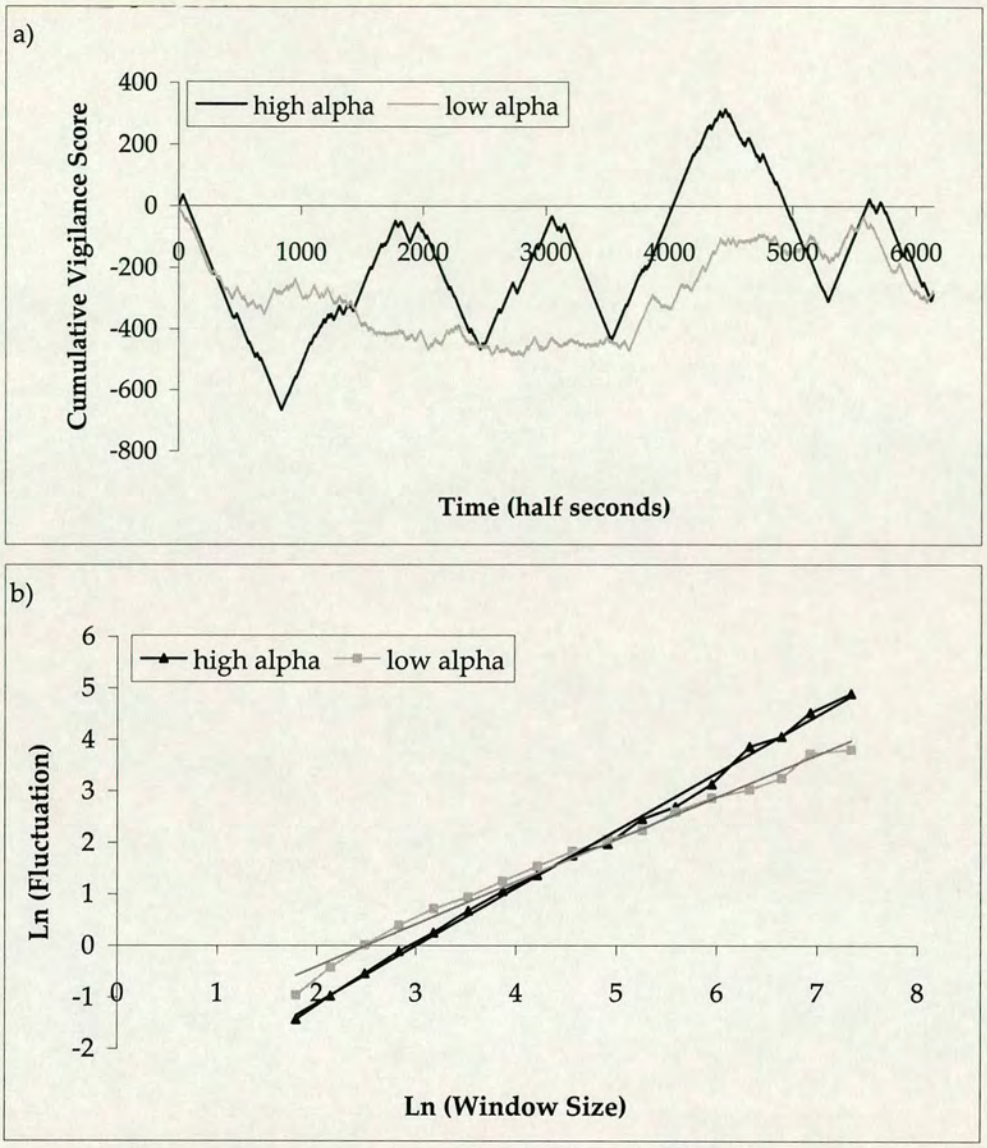


Figure 4-3: Extremes of fractal complexity in the vigilance data

a) The two original time series for the birds with either the highest (black line) or lowest (grey line) Vα (from all observations), representing the extremes of high and low long-range autocorrelation respectively.
b) Fluctuation value against window size for the same two series, plotted on a double log scale. The series (black triangles) with the highest Vα (lowest behavioural complexity) is characterised by the equation Y=1.12x – 3.348 (black line), R^2 = 0.997. The series (grey squares) with the lowest Vα (greatest complexity) is characterised by the equation Y=0.82x – 2.045 (grey line), R^2 = 0.99.

4-4. Discussion

The pattern of fluctuation between vigilant and non-vigilant behaviours and between activity and inactivity showed long-range autocorrelation, such as that found in minnow reproductive behaviour and chimpanzee social behaviour (Alados & Weber 1999; Alados & Huffman 2000). This means that the behavioural patterns are persistent from moment to moment and that they occur non-randomly. In the case of vigilance the complexity of the fluctuation pattern increased in the mildly stressful situation of placement in a novel arena or after restraint and blood sampling, while blood sampling alone caused no change. The total time spent vigilant also increased in the novel arena but not in the other situations. This indicates that the behavioural pattern is qualitatively but not quantitatively altered following restraint. DFA therefore reveals information about the nature of behavioural expression, which can alter independently of the total amount of behaviour shown in any given period. This is well illustrated by the plots from two birds showing extremes of complexity but similar final cumulative vigilance scores (Fig. 4-3a), indicating very similar total duration of vigilance. This suggests that using DFA in addition to traditional analyses can provide valuable additional information about behavioural organisation. Furthermore, the fact that the DFA method can be applied to simple behavioural transitions means that subjective interpretation of behaviour is reduced to a minimum.

In contrast to the alterations seen in vigilance organisation, the fractal structure of the temporal pattern of activity did not alter in any of the observation situations when compared to undisturbed home pen observations. It could well be that there are specific reasons why vigilance should change when other behavioural systems do not. The small yet significant shift towards a random fluctuation pattern, in the novel arena and following restraint compared to that shown in the home pen, could represent an adaptation to perceived threat; a random vigilance pattern being supposedly more difficult for a watching predator to 'work out' (Pulliam 1973). However, it is debatable whether such a small change in behaviour could be viewed as an adaptation. In threatening situations the most adaptive response would appear to be an increase in total vigilance – as was apparent in the novel arena. Alternatively, the relatively small amount of walking shown by the birds in their small pens may have decreased the chances of observing a treatment difference in

activity. The only alteration seen in walking was a small increase in the mean length of each walking bout in the novel arena, which could simply reflect the greater space available in the arena compared to the home pen. Activity was much more randomly organised than vigilance (as indicated by the lower alpha values) and this may reflect a genuine difference in the organisation of walking and vigilance. The difference could, however, also be due to an artefact of the method – walking may be recorded as being randomly organised purely because so little walking was seen. This possibility is partially supported by the fact that the alpha value was significantly correlated to the total duration of walking (i.e. birds that spent longer walking had a less random walking pattern). This may mean that, in this experiment, the fractal description of activity is a less reliable measure of behaviour.

It could be argued that, since there were alterations in other behavioural measures in the test situations (Table 4-4), DFA does not provide any extra information. Exposure to some or all of the stressful treatments reduced the frequencies of stretching, a low priority 'comfort' behaviour (Black & Hughes 1974), ground scratching and drinking. However, since there is no guarantee that these particular behaviours will occur in any given short observation they are considered unlikely to represent reliable indicators of stress. Furthermore, although the home pen, blood sampling and restraint plus blood sampling treatments provided a putative gradation of stressful stimulation, observed alterations in stretching and scratching did not differentiate between them.

In contrast to previous applications of fractal analysis to behavioural patterns (Alados et al. 1996; Alados & Weber 1999; Alados & Huffman 2000), where stress reduced behavioural complexity, an increased behavioural complexity was found here, indicating a move towards a more random pattern. This apparent inconsistency probably reflects differences in the nature and duration of the stressors involved. On the one hand, Alados and her colleagues have studied more chronic situations (pregnancy, parasitic load, disease and lead exposure) that impose an energetic cost on the animals but may not necessarily involve any negative mental states such as fear or distress. Conversely, the treatments used in the present study were short-lived and putatively induced fear and an associated mild distress in the animals. In this case the increased complexity seen in behaviour might be interpreted in terms of a more active response. It is not yet known whether

this effect (increased complexity) is peculiar to the particular stressors used in our experiment or to acute stressors in general.

The stressors used here are considered to be relatively mild and not to represent a major welfare concern in themselves. Despite this our results have important implications for animal welfare science. Although many of the welfare insults associated with housing systems are chronic in nature their impact on an animal's ability to cope with short-term stressors is important (e.g. Boissy et al. 2001). For instance, chronic elevation of plasma corticosterone can increase underlying fearfulness, an increased readiness to respond can reduce the response threshold as well as result in exaggerated responses, and sensitization of the stress response is thought to contribute to the development of pathological anxiety, hyperexcitabilty and abnormal behaviours (Rosen & Schulkin 1998; Jones et al. 2000). Thus increased reactivity to acute stressors may indicate the experience of negative mental states that in turn represent a welfare concern. In addition to this, repeated or exaggerated stress responses themselves may have a physically or cognitively debilitating effect on an animal (e.g. through the long-term effects of increased glucocorticoid levels and 'allostatic load': McEwen & Stellar 1993; de Kloet et al. 1998).

Animals in intensive agricultural husbandry systems are regularly exposed to acute disturbances that could act as stressors (e.g. novel stimuli, human contact, loud noises etc) and their ability to cope with these is an important determinant of welfare. Measuring the magnitude and duration of any change in behavioural complexity (i.e. the degree of deviation from normal, undisturbed behaviour) could potentially provide a non-invasive means of assessing coping success. The premise would be that coping success is inversely related to deviation from normality. Indeed, Wechsler (1995) suggests, "an animal with difficulty in coping may increase the duration, frequency or intensity of coping behaviour" i.e. the deviation from normality would be greater. Although in the short term an animal may show a distinct response to a stressor (including greatly increased vigilance), following this stage DFA may allow lingering alterations that may indicate the success or otherwise of coping strategies, to be measured.

4-5. Conclusion

As noted in the introduction, to be able to compare different stressors or environments it is necessary to measure responses on a single non-specific scale. The results provide preliminary evidence that a fractal analysis methodology could provide such a scale, allowing general statements about the organisation of different behavioural patterns to be made.

The present results show that novel aspects of poultry behaviour can be measured using the DFA method. That the pattern of fluctuation between vigilant and non-vigilant behaviours altered under mildly stressful or fear inducing situations indicates that this method may have a promising role as a non-invasive measure in overall assessments of welfare. First though, the effects of other changes in the environment (e.g. group size, light levels) or the state of the birds (e.g. hunger, oviposition) that could alter behavioural complexity need to be investigated.

Chapter Five

The effects of a chronic-intermittent stressor regime on welfare-related indices and fractal behavioural complexity in laying hens

Abstract

In modern poultry husbandry systems laying hens may be exposed to repeated stressors over the course of their life. To investigate the effects of such a chronic-intermittent exposure, adult ISA Brown laying hens were repeatedly exposed to numerous varied acute stressors over two ten-day periods (S-1 and S-2) with the aim of creating a biologically costly stress state of increased allostatic load in the animals. The degree of stress imposed on the animals was assessed by comparing treated animals to controls on measures of behaviour, body weight, egg production and quality, plasma corticosterone concentration and the heterophil to lymphocyte (H/L) ratio. Behaviour was analysed using both standard techniques and the fractal analysis method of Detrended Fluctuation Analysis (DFA).

Stress treatment birds lost weight during S-1 ($P = 0.023$). Food intake was also reduced relative to controls in S-1 ($P = 0.09$) and significantly in S-2 ($P = 0.014$). The average egg weight also decreased during the stress periods ($P = 0.051$) and significantly in the period following the end of the stress ($P = 0.037$). These changes in body weight, food intake and egg weight indicate that the stress group birds may have been stressed for at least part of the treatment duration. However, the degree and consistency of induced stress is uncertain. Corticosterone concentrations were actually larger in the control group following S-1 ($P < 0.01$) and the H/L ratio did not differ between the two groups at any time point. Standard behavioural measures also did not differ between the two groups. A DFA on either activity or vigilance patterns did not reveal any difference in behaviour between control and stress group birds. There was a highly significant negative relationship ($r = -0.72$, $P = 0.004$) between the complexity of activity and the H/L ratio in one of the four observations. This result deserves to be pursued but cannot be considered to provide enough evidence for stating that the fractal behavioural complexity of chickens alters under chronic-intermittent stress.

5-1. Introduction

The question of if, and when, animals might suffer from chronic stress and alternatively in what circumstances they can adequately adapt to their housing and husbandry is fundamental to animal welfare science. However, to test methods for measuring chronic stress it is necessary to have a reliable standard model in the form of a set of conditions that consistently elicit stress. Ladewig (2000) has recently suggested that repeated exposure to diverse acute stressors might provide a useful model for studying long-term effects of stress in animals. Such a stressor regime may be closer to the reality of domestic animals' experience. While acute stress responses are transient and may ideally help the animal to adapt to a challenge (Wiepkema & Koolhaas 1993), in the unnatural captive environment they may be ineffective in removing the source of challenge or allowing the animal to cope. Equally, the captive environment can prove to be a source of repeated acute stressors and can create anxious animals that perceive challenges where none exist (Rosen & Schulkin 1998). In these cases a chronic stress state may emerge. Despite chronic stress being a commonly used term there is no clear definition of what constitutes chronic stress. Chronic stress might be considered to be that which extends over periods of days or longer. However, any chosen time frame is arbitrary and it may be more productive to view chronic stress as occurring when the body's biological defences spill over from a normal, beneficial, stress response to an abnormal and deleterious stress state (McEwen & Stellar 1993; de Kloet et al.1998).

In Broom's often-quoted definition of welfare, what is important is the animal's success at coping with the stressors it faces (Broom 1986, 1996). However, apparently successful coping may come at a cost. Situations that chronically or repeatedly stress an animal are energetically costly (e.g. Laugero & Moberg 2000ab). When multiple stressors exist, these can have additive or synergistic effects (McFarlane et al. 1989ab; McFarlane & Curtis 1989). In stress research such a biological cost has been called an allostatic load (AL) (McEwen & Stellar 1993); a concept developed from Sterling and Eyer's (1988) introduction of the term allostasis. AL is the accumulated cost that results from repeated physiological alterations (e.g. repeated responses in a high stress reactive individual), and/or elevated physiological set points or increased physiological 'effort' required to maintain a set point (Seeman et al.1997; McEwen & Stellar 1993). Under increased AL, organisms, although apparently coping with their situation, are

more vulnerable to acute events. In mice, a chronic variable stress regime increased the sickness response and weight loss induced by a standard immune challenge (Tannenbaum et al. 2002). The state of increased allostatic load might be seen as being synonymous with Moberg's (2000) description of 'sub-clinical' or 'pre-pathological' stress states, where the appearance of normality masks an increased vulnerability to new challenges; either to homeostasis, or to psychological well-being (McEwen 2000a).

If on-going behavioural parameters that correlated reliably with the degree of allostatic load could be identified it could aid the assessment of chronic stress in captive animals. A series of studies by Alados and colleagues (Alados et al. 1996; Alados & Weber 1999; Alados & Huffman 2000) have shown that in situations that could be thought of as involving increased allostatic load, (pregnancy, parasitism or disease), the fractal complexity of behavioural patterns decreased. It was suggested that under increased energetic demand animals scale-down the complexity of their activity, while maintaining total activity (Alados et al. 1996). With this theory in mind it is possible that fractal analysis of behaviour could provide a useful indicator of chronic stress. The current experiment attempted to investigate the effects of exposure to a regime of chronic intermittent stressor on the fractal complexity of behavioural patterns. The expectation, based on Alados' studies, is that behaviour would decrease in complexity under chronic stress.

Although reliable behavioural models of chronic stress do not exist in livestock, such models are common in the biomedical field. Many of the stressors used in animal models of depression, for instance, have noticeable parallels to the experience of farmed animals. The form of stress regime used here was inspired by the Chronic Mild Stress (CMS) model of depression (Willner et al. 1992; Willner 1997bc). In the CMS model, rats are exposed to a series of mild stressors, typically for several weeks. Stressors used include: food or water deprivation, altered lighting, cage tilt, group housing, soiling of the cage, reduced temperature, intermittent white noise, odour and novelty (Willner et al. 1987). While the use of the CMS protocol as a model of depression has been questioned (Forbes et al. 1996; Matthews & Reid 1998; Murison & Hansen 2001) it does appear to produce a reliable costly stress, with reductions in body weight or decreased growth rate typical (Forbes et al.1996; Willner et al. 1996; Willner 1997b). It is felt that, in the CMS model, the unpredictable and uncontrollable application of varied stressors decreases the ability of the animal to adapt to each

stressor individually (Rodríguez-Echandía 1988). (Note, however, that this theory has been questioned: Lin and colleagues (2002) found habituation to restraint occurred within their version of the CMS regime). The irregular and continuous combination of environmental and social stressors used in the model may have relevance for studies of chronic stress, based on the theory of allostatic load, in captive animals. The aim of this experiment was to develop a model of chronic-intermittent stress in poultry on the basis of this hypothesis. A secondary aim was to test the validity of fractal analysis of behaviour as an indicator of stress in chickens.

To accurately assess the biological effects of the stressor treatment and to provide as rigorous as possible a test of fractal analysis, many other different measures were taken. Blood samples were taken and analysed for corticosterone concentration and the heterophil to lymphocyte (H/L) ratio, both of which are standard measures of stress in poultry (Gross & Siegel 1983; Jones 1989b; Maxwell 1993; Siegel 1995). Measurements of food intake, body weight, egg production, egg weight, egg breaking strength and eggshell thickness were also taken, since these parameters have previously been shown to alter under stress in poultry (Hughes & Black 1976; Gross & Siegel 1981; McFarlane et al. 1989a; Johnson et al. 1991; Bollengier-Lee et al. 1998; Puvadolpirod & Thaxton 2000a; El-lethey et al. 2000, 2001). Finally, tonic immobility duration was measured to assess whether the treatment caused any alteration in underlying fearfulness (Jones 1996).

5-2. Animals, materials and methods

5-2-1. Animals

The experimental animals were 48 adult ISA Brown hens housed in pairs. These birds were reared singly in cages to 52 weeks of age and then transferred to small metal floor pens (105x100cm) bedded with wood shavings. The experiment started 18 days after the transfer from cages to the floor pens. This period allowed habituation to the pens and the presence of experimenter. The birds were kept on a 14:10 light-dark cycle, with lights coming on at 6 am. Apart from where dictated by experimental treatment, all birds were allowed *ad libitum* access to food (standard adult layer pellets) and water.

5-2-2. Treatments

Birds were randomly allocated to two treatments ('**stress**' or '**control**') with pens alternating between treatments across the room (Figure 5-1). Within each pair of birds, one bird was randomly chosen as the test bird and the other acted as a companion only. In the control group, birds were left as undisturbed as possible. Birds within the stress group were exposed to two identical 10-day stress periods (**S-1** & **S-2**), during which the test bird was exposed to numerous acute stressors each day. Some additional stressors were applied concurrently to provide novel combinations (see Table 5-1). This was an attempt to reduce the likelihood of habituation to individual stressors occurring. Descriptions of each of the individual stressors used are given in Table 5-2. The initial sample size was 12 in each treatment, however this was reduced during the course of the experiment due to feather pecking in some pens and also to ill health in two birds. The sample size at each point is indicated in each statistical test.

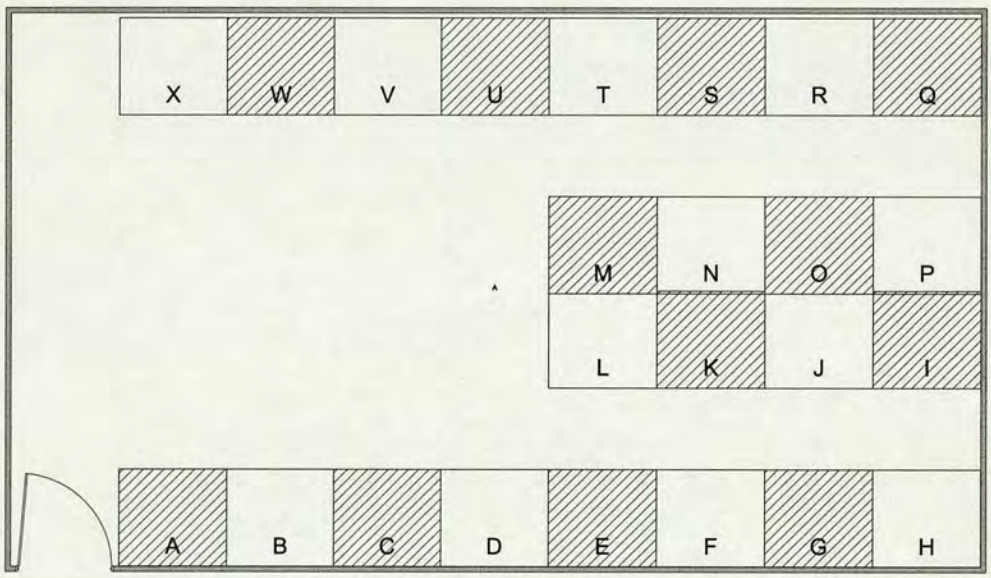


Figure 5-1: Diagram of experimental room layout

Each lettered square represents a pen of two birds. Shaded pens are those in the stressor treatment, blank one are those in the control treatment. The pens had solid sides and backs. The bottom half of the front of each pen was solid and the top half consisted of a wire grating, allowing the birds some limited view out of the pen.

Table 5-1: Stressor schedule

Day	Stressor 1	Stressor 2	Stressor 3	Stressor 4	Stressor 5
1	Isolation	Flash	Transport	Crush cage	
2	Food removal (9am – 4pm)	Novel arena	Sudden noise	Heat	
3	Cone restraint	Transport	Isolation	Novel object	
4	Heat	Transport	Crush cage	Fan	
5	Isolation	Water spray	Food removal (Noon–5.30pm)	Social	
6	Transport	Water spray	Cone restraint		
7	Isolation	Sudden noise	Crush cage	Fan	Food removal (9.30am-5pm)
8	Isolation	Water	Heat	Social	
9	Food removal (10am-5pm)	Transport	Transport	Isolation	Novel object
10	Cone restraint	Heat	Crush cage	Water	

Lack of a dividing line indicates that the stressors were applied concurrently, e.g. on day three novel objects were applied to the bird when in isolation. This schedule was repeated exactly in the two stressor-periods: S-1 and S-2.

Table 5-2: Description of the experimental stressors

Main stressors	Description
Isolation	The test bird was removed from its pen and isolated in another pen in a different room for one hour.
Heat stress	Both test and companion birds were placed, within transport crates, in a controlled climate chamber operating at an average of 31.5º C and 55% relative humidity for one hour.
Mechanical restraint	The test bird was removed from the home pen and restrained in a crush cage (see Chapter Four for details)for ten minutes.
Restraint cone	The test bird was removed from the home pen and placed in a restraint cone for ten minutes. The restraint cone is a metal inverted cone with a hole in the bottom. The bird is inverted and placed in the cone so that its head comes out the hole in the bottom. If the bird struggled out of the cone it was replaced and the restraint continued.
Food removal	The food hopper was removed from the pen for a variable duration (see Table 5-1).
Social stress	The pairs of birds (test and companion) were placed on one side of a small metal pen (as home pen) that had been divided into two with a wire mesh frame. Another pair of unfamiliar birds was placed on the other side of the mesh.
Open field	Both birds were moved to an unfamiliar larger arena (140x140cm) for one hour.
Transport	Both treatment and companion birds were removed from their home pen and placed in standard transport crates (four or five birds per crate) and wheeled manually on a trolley for a period of 15 minutes.
Additional stressors	
Water spray	The water spray treatment was used in combination with isolation, crush cage or transport. The test bird was squirted five times in a 20-minute period with a full 20-ml syringe of water from a distance of approximately 1m.
Sudden noises	A sudden alarming noise was created by dropping a 3m steel rod from waist height onto a concrete floor. This was repeated five times in a 30-minute period. This was combined with novel arena. When birds were in the small isolation pen a sudden noise disturbance was created by pulling a pitchfork along the front of the cage five times in a 30-minute period.
Fan	A 60watt fan was placed beside the crush cage and directed at the bird.
Novel objects	Various novel objects were used in combinations with the isolation or open field stressors. These were; rubber boot, traffic cone, yellow watering can, blue plastic sheet, large white plastic disk. Birds were exposed to each object for up to ten minutes.
Flash	The flash treatment was used in combination with transport. The birds within the crates were taken into a darkened room at the start of their transport period and exposed to five repeated firings of a standard photographic flash bulb.

5-2-3. Measurements

5-2-3-1. Body weight and Food intake

The body weight of the test birds was measured four times over the course of the experiment (see Figure 5-2). The amount of food eaten during the two stress periods and in the 'recovery' period following the end of S-2 was measured in each pen.

5-2-3-2. Eggs

During the experiment all the eggs that were laid were counted and weighed. Egg production was expressed on a percentage scale where 100% equals one egg laid by every hen, every day. All the egg variables were analysed in batches of five days (see Figure 5-2).

On two occasions the breaking strength of all eggs laid was measured. To assess breaking strength a quasi-static compression fracture test (Hunton 1987) was carried out using a Lloyd Instruments LRX 50 materials tester, fitted with a 100Newton load cell. For this test the egg is placed between two parallel surfaces that move together at a fixed rate (20 mm/minute). The measure of strength is the force required to cause a crack and various other strength related parameters are calculated by the instrument on the basis of these data. Measurements of shell thickness (the mean of two measurements around the equator of the egg, using a micrograph), length and width were also made on these eggs.

5-2-3-3. Behaviour

Four one-hour video recordings in undisturbed conditions were made on each test bird, with a camera positioned at the front of the metal pen. The first observation was prior to S-1, the second between S-1 and S-2, and the third and fourth following S-2 (one immediately after S-2 and one after a 'recovery' period of 11-13 days). Recordings were made in the afternoon to limit the interference of laying behaviour. Four observation times were used (12noon-1pm, 1.20pm-2.20pm, 2.40pm-3.40pm and 4pm-5pm) with two pens being recorded at each time point (one control bird, one stress bird). The whole set of observations took three days.

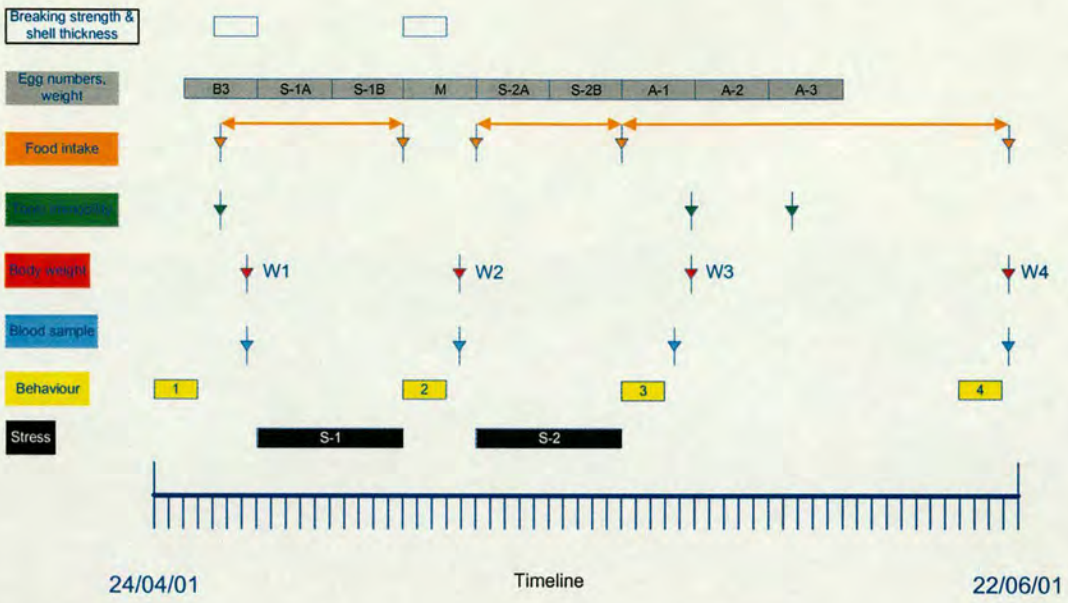


Figure 5-2: Timeline of stressor application and experimental measures

The experiment ran for sixty days. Each division on the timeline is equal to one day. The timeline is shown from the first behavioural observation onwards. For the egg and food intake data the experimental unit is the pen rather than the individual test animal. The egg data were divided into five-day long batches: B1-B3 = Before 1, 2, & 3; S-1A & S-1B = first and second half of first stress period; M = Middle period between S-1 and S-2; S-2A & S-2B = first and second half of second stress period; A1-A3 = After 1, 2 & 3. Some eggs (Batches B1 & B2) were collected and weighed prior to the first observation, so are not shown on this diagram.

5-2-3-4. Corticosterone concentration and leucocyte counts

A four-millilitre blood sample was taken from the brachial vein, on four occasions: prior to the first stress period, between S-1 and S-2 (five days after the end of S-1) and twice after S-2 (five and 28 days after the end of S-2). All four blood-samples were analysed for leucocyte counts, while only the first three samples were analysed for corticosterone concentration. The test birds were sampled in a pre-determined random order but with the same order used each time. All blood samples were taken on the day following the last behavioural observation starting with the first bird at 2pm. Samples were taken in the afternoon to avoid any rise in corticosterone concentrations associated with oviposition (Beuving & Vonder 1977, 1981; Gibson et al. 1986). The blood for subsequent corticosterone assay was collected into a lithium-heparin tube and then centrifuged at 1500g and 10°C for ten minutes. Plasma was pipetted off and

frozen for later analysis of corticosterone concentration using a commercially available radioimmunoassay kit. Before the blood was placed in the heparin tube, a small amount was smeared onto a slide. These slides were stained the same day in a May-Grunwald-Giemsa stain. For measurement of leucocyte profiles (heterophils, lymphocytes, monocytes, eosinophils and basophils) slides were relabelled so that the observer was blind to treatment and sample. One hundred cells were counted from each slide. The H/L ratio was calculated by dividing the number of heterophils by the number of lymphocytes.

5-2-3-5. Tonic immobility (TI)

TI duration was measured three times over the course of the experiment, once prior to S-1 and then five and 12 days after S-2 (see Figure 5-2). The test order alternated between test and control birds, in a pre-determined random order but with the same order each time. Birds were restrained on their back on a cloth-covered cradle by the experimenter, who placed one hand over the bird's sternum and the other over the head – guiding the head down over the edge of the cradle and shielding the eyes. After 15 seconds in this position the experimenter released the bird and if it did not immediately show a righting response the duration until it did so was measured. If the bird did right itself then the procedure was repeated. The number of necessary inductions (to a maximum of five) and the duration of immobility (to a maximum of 20 minutes) were recorded for each bird. The observer remained standing motionless by the cradle during immobility.

5-2-4. Data analysis

5-2-4-1. Presentation

Unless otherwise stated, data values are presented as means and standard deviations, with the values for the stress group (S) presented first and control (C) second. In most cases companions are ignored in the analysis so references to stress and control groups refers only to the test birds. However, for the feed intake and egg production data the experimental unit is the pen rather than the test animal. Many of the tables contain results that did not show any treatment related differences. These tables are included in appendix tables A5-1 to A5-13.

5-2-4-2. Multiple comparisons

Where necessary, P values are corrected for multiple comparisons using the Bonferroni correction (Darlington & Carlson 1987; Curtin & Schulz 1998). Briefly, where multiple comparisons are made the P value is altered to take into account the fact that likelihood of making a type one error (false positive) is increased. For instance, taking the standard P value cut-off of 0.05% for significance, one test result out of every twenty will be found to be significant purely by chance. There is no standard practice for deciding the stringency of a Bonferroni correction. Correcting all the tests within a whole experiment would greatly increase the chance of making a type two error (e.g. creating false negatives). Here it was decided to correct the P values within each variable taking into account the number of repeated measures of that variable (e.g. behavioural values are corrected by a factor of four to reflect the four repeat observations) as it was felt that this represented a suitable balance between the risks of making type one and type two errors. Where no significant values were obtained prior to any correction the original values are displayed. When a P value, prior to correction, less than 0.1 was obtained the corrected P value is shown in addition to the original, in the format: (original/corrected).

5-2-4-3. Behaviour and tonic immobility

Behavioural observations were made according to an ethogram (Table A5-1) based on that used in experiment one (Chapter Four), with some additions. Durations and frequencies of behavioural parameters were calculated in Keytime (Deag 1993). The data were also analysed using Detrended Fluctuation Analysis (DFA), as described in Chapter Four. The calculated DFA values for vigilance and activity are referred to as $V\alpha$ and $A\alpha$ respectively. As in Chapter Four, behavioural analysis was restricted to 3072 seconds.

Differences between the two treatment groups at each time point were tested for using a Two-sample t-test (were necessary using transformed data) or a Mann-Whitney test, where appropriate (i.e. where data transformation to satisfy parametric requirements was not possible). To assess the consistency of individual leucocyte values over the four samples Kendall's coefficient of concordance was calculated (Siegel 1956). The coefficient, W, represents the degree of association between the rankings of each individual over the four observations. W ranges from zero (where

there is absolutely no association) to one (where there is perfect association). Comparisons of TI duration required log ten transformations.

5-2-4-4. Eggs

To avoid pseudoreplication, a mean value for egg weight over all the eggs laid in each pen over each five-day period was calculated (i.e. there was one value for each pen, rather than using all eggs in each statistical comparison). For the breaking strength data, all the eggs laid in each pen over a three-day period prior to the start of stress exposure and for the four days between S-1 and S-2 were measured. This was a total of 88 eggs and 90 eggs respectively. A mean value for each pen for each parameter was calculated and this is the value used in the analysis.

5-2-4-5. Comparison of DFA values with other data

Given that there was considerable variation in most variables, following the initial analysis it was decided to analyse the results for all birds ignoring treatment groups. The rationale behind this was that some of the birds in the control group may have been stressed and there may well be a degree of variability in the severity of any stress in the 'stress' group. Therefore the DFA values for all the birds at each time point were compared with the appropriate values for other variables.

5-3. Results

5-3-1. Body weight

There was no significant difference in body weight between the two groups at any of the four weighing points (Table A5-2). However, within groups over the course of S-1, weight significantly decreased in the stress group (W2-W1= -80.5g ± 92.9: One-sample t-test, t=-2.74, P=0.023, df=9) but not in the control group (W2-W1=17.4 ±174.6, t=0.3, P=0.77, df=8). Over S-2 there was a significant increase in weight within the stress group (W3-W2=58± 76.8, t=2.39, P=0.041) but no significant alteration in the control group (W3-W2=10.4±178.1, t=0.16, P=0.87). Over both stress periods combined there was no significant alteration in weight in either the stress group (W3-W1= -22.5±115.6, t=-0.62, P=0.55) or the control group (W3-W1= 47±167.3, t=0.79, P=0.45). Over the 'recovery' period there was no significant change in weight in the stress group (W4-

W3= 14.7±117.1, t=0.4, P=0.7) and a trend towards an increase in the control group (W4-W3= 44.4±59.7, t=2.1, P=0.074).

5-3-2. Food intake

Over S-1, stress group birds ate less than control birds although the difference between the two groups only approached significance (S=1681g ± 559 vs. C=2095g ±413: Two-sample t-test, t=-1.79, P=0.093, df=16). These totals translate to an average daily intake per pen of 140.1g in the stress group and 174.6g in the control group. Over S-2 there was a significant difference between the two groups (S=2157g ±375 vs. C=2596g ±279.7: t=-2.75, P=0.014, df=16), with the control pens consuming more food on average. These totals translate to an average daily intake per pen of 196.1g in the stress group and 236g in the control group. The daily food intake was significantly higher over S-2 compared to S-1 for both stress (S-2-S-1=61.4 ±46.8, One-sample t-test; t=3.93, P=0.0044) and control groups (S-2-S-1=58.5 ±35.2: t=4.72, P=0.0023). Over the whole recovery period there was no difference between the two groups (S=5081g ± 723 vs. C=5302g ± 581: t=-0.7, P=0.49, df=16). These totals translate to an average daily intake per pen of 181.5g in the stress group and 189.5g in the control group.

5-3-3. Egg measures

The egg production of the two groups did not differ statistically in any of the time periods (Table A5-3). For egg weight (Table A5-4) there was no difference between the two groups during periods B1, B2 or B3 or on average over the whole period (S=63.9±3.94 vs. C=65.2±3.16: t =-0.75, P=0.47, df=17). There was no difference between the two groups for batch S-1A. However, there was a significant difference, prior to Bonferroni correction, between the two groups over S-1B. There was no difference in egg weight in the gap period (M) between S-1 and S-2 and only a trend towards a difference between the two groups over S-1A and S-1B. Over the whole stress period there was a trend towards a difference between the groups, with the stress group producing lighter eggs (S=62.5±1.89 vs. C=65.2±3.68: t=-2.1, P=0.051, df=17). There was a significant difference between the two groups over period A1, prior to Bonferroni correction but not A2. Over the whole period following the stress periods hens in the stress group laid lighter eggs on average (S=63.5±3.5 vs. C=67.2±3.24: t=-2.28, P=0.037, df=16).

There were no significant differences between treatments for the egg strength parameters or for shell thickness (Table A5-5). The only statistically significant result found, prior to Bonferroni correction, was that the stress group laid significantly narrower eggs prior to the start of the stress exposure.

5-3-4. Behaviour - Standard measures

There was no difference between the two treatment groups at any time point in the duration, frequency or bout length of vigilance (Table A5-6), or activity (Table A5-7), or in the durations of any of the individual behavioural states (Table A5-8) or frequency of behavioural events (Table A5-9).

5-3-5. Behaviour - Detrended fluctuation analysis

Over all observations and both treatments the values for $V\alpha$ range from 0.594 to 1.317. There was no difference in $V\alpha$ between the two groups for the first (S=0.95 ± 0.07 vs. C=0.95 ±0.08: t= -0.08, P=0.94, df=17), second (median: S=0.99 vs. C=0.97: Mann-Whitney, W=111, P=0.39), third (median: S=0.94 vs. C=0.93: W=93, P=0.89) or fourth (median: S=0.93 vs. C=0.95: W=87, P=0.81) observation.

Over all observations and both treatments the values for $A\alpha$ range from 0.546 to 1.17. There was no difference in $A\alpha$ between the two groups for the first (S= 0.76±0.05 vs. C=0.76±0.06: t=-0.08, P=0.94, df=17), second (median: S= 0.73 vs. C=0.78: W=82,P=0.15), third (S= 0.78±0.1 vs. C=0.79±0.08: t=-0.25, P=0.81, df=16) or fourth (S=0.77±0.04 vs. C=0.76±0.05: t=0.57, P=0.58, df=15) observation.

Further analysis and description of these data is presented in section 7-2-3.

5-3-6. Corticosterone concentration

Prior to the start of the stress treatment there was no difference in plasma corticosterone concentration between the groups (S=1.8ng/ml ±0.94 vs. C=2.4ng/ml ± 1.94: Two-sample t-test (log ten transformed), t=-0.81, P=0.43, df=17). However, following S-1 the corticosterone concentration in the control group was significantly greater than that in the treatment group (S=1.65ng/ml ± 0.51 vs. C=2.33ng/ml ± 0.39: t= -3.2, P=0.0056, df=16). Following S-2, there was no difference between the groups (median: S= 2.2 ng/ml vs. C=2.0 ng/ml: Mann-Whitney, W=106, P=0.35).

There was no difference between the two groups in the sampling time, for the second (median: S=83seconds vs. C=95seconds: M-W, W=86, P=1) or third (median= 83.5seconds vs. 80seconds: M-W, W=102, P=0.56) sample. (The time taken from the bird leaving the pen to blood removal was not recorded for the first sample). There was also no relationship between the time taken to take the sample and the measured concentration of corticosterone (second sample, r=0.043; third sample, r = 0.33). Corticosterone concentration does not correlate with sample order in the first (r=0.2), second (r=0.17) or third (r= 0.2) sample. So birds sampled later did not have higher corticosterone concentrations.

5-3-7. Leucocyte profiles

There was no difference in the percentages of the five different leucocytes or in the H/L ratio between the two treatment groups at any sample point (Table A5-10). Within birds the percentages of heterophils, lymphocytes and the H/L ratio were consistent across samples in the control group but not in the stress group (Table 5-3). Conversely, the percentages of basophils and monocytes were consistent in the stress group but not the control. Following correction for multiple comparisons there was no correlation between the different leucocyte parameters and the standard measures of vigilance (Table A5-11) or activity (Table A5-12).

Table 5-3: Leucocyte consistency across samples

Group	Heterophil %	Lymphocyte %	H/L ratio	Monocyte %	Eosinophil %	Basophil %
All N=18	KC=0.32 P=0.18	KC=0.36 P=0.1	KC=0.32 P=0.19	KC=0.44 P=0.017	KC=0.3 P=0.25	KC=0.49 P=0.011
Stress only N=10	KC=0.15 P=0.79	KC=0.29 P=0.32	KC=0.22 P=0.56	KC=0.5 P=0.023	KC=0.3 P=0.26	KC=0.56 P=0.012
Control only N=8	KC=0.57 P=0.026	KC=0.50 P=0.048	KC=0.52 P=0.043	KC=0.4 P=0.1	KC=0.19 P=0.55	KC=0.28 P=0.31

KC = Kendall's coefficient of concordance

5-3-8. Tonic immobility

Birds in the control group had a longer mean immobility duration in the first test (S=98seconds ± 105.1 vs. C=228.8seconds ± 193: Two-sample t-test: t= -2.24, P=0.038, df=17). In the second test five days after the end of S-2, there was no difference between the groups (S=261seconds ± 247.2 vs. C=166seconds ± 93.5: t=0.21, P=0.84, df=16). Although the duration of immobility in the stress group increased on average between tests one and two, while in the control group the duration decreased, the change was not significantly different from zero in either the stress group (T2-T1=164±289.9: One-sample t-test, t=1.78, P=0.11) or in the control group (T2-T1=-63±212: t=-0.84, P=0.43). In the third test there was no difference between the two groups (S=345seconds ±454 vs. C=248seconds ± 201.3: t=-0.11, P=0.91, df=16).

5-3-9. Relationship between DFA values and other data

5-3-9-1. DFA versus corticosterone concentration.

For all birds and all observations there was a trend towards a negative relationship between the $V\alpha$ and the corticosterone concentration in the following sample (r=-0.24, P=0.075). This means that birds with higher corticosterone concentrations tend to a lower $V\alpha$ value indicating a more random structure to their vigilance behaviour. However, it is highly debatable whether this is a real or biologically significant relationship. Within each of the individual observations there was no relationship (one: r=-0.2, P=0.42, two: r=-0.29, P=0.24, three: r=-0.35, P=0.16). There was no relationship between $A\alpha$ and the corticosterone concentration, either for all samples/observations together (r=0.059, P=0.67) or for the individual samples/observations (one: r=0.286, P=0.24, two: r=-0.075, P=0.78, three: r=-0.052, P=0.84).

5-3-9-2. DFA versus change in body weight

There were no relationship between change in body weight at any point in the experiment and the associated observation (e.g. the degree of weight loss in individual birds does not relate to $V\alpha$ or $A\alpha$) (Table A5-13).

5-3-9-3. DFA versus individual leucocyte percentages and the H/L ratio

The only significant relationship found for vigilance was between Vα and the percentage of monocytes in the third observation (Table 5-4). There were highly significant correlations between the Aα and the H/L ratio, the percentage of lymphocytes and the percentage of monocytes in the third observation/sample (Table 5-5). There were also significant correlations in the same observation/sample between the DFA value and the percentage of heterophils and eosinophils. Even with the most stringent correction for multiple comparisons the relationship between H/L ratio and Aα is significant.

Table 5-4: Relationship (correlation percentage (p-value)) between Vα and the percentage of individual leucocytes and the heterophil to lymphocyte ratio, in each observation

	Observation One	Observation Two	Observation Three	Observation Four	All
H/L RATIO	-7 (0.79)	-25 (0.30)	30 (0.22)	31 (0.24)	11 (0.36)
HETEROPHIL %	-9 (0.72)	-25 (0.31)	18 (0.49)	24 (0.36)	6 (0.61)
LYMPHOCYTE %	9 (0.7)	21 (0.39)	-22 (0.39)	-22 (0.4)	-8 (0.49)
MONOCYTE %	2 (0.94)	14 (0.57)	58 (0.01/0.04)	16 (0.55)	27 (0.02/0.08)
EOSINOPHIL %	11 (0.65)	17 (0.48)	0.1 (1)	-17 (0.51)	6 (0.65)
BASOPHIL %	-16 (0.53)	-24 (0.32)	-26 (0.3)	1 (0.97)	-14 (0.22)

Table 5-5: Relationship (correlation percentage (p-value)) between Aα and the percentage of individual leucocytes and the heterophil to lymphocyte ratio, in each observation

	Observation One	Observation Two	Observation Three	Observation Four	All
H/L RATIO	-29 (0.22)	20 (0.42)	-72 (0.001/0.004)	7 (0.78)	-19 (0.11)
HETEROPHIL %	-31 (0.2)	16 (0.53)	-58.7 (0.01/0.04)	6 (0.82)	-15 (0.2)
LYMPHOCYTE %	38 (0.11)	-19 (0.43)	70 (0.001/0.004)	-0.3 (0.99)	18 (0.12)
MONOCYTE %	-16 (0.51)	14 (0.57)	-67 (0.002/0.008)	-3 (0.9)	-15 (0.21)
EOSINOPHIL %	-51 (0.025)	13 (0.6)	-47 (0.048/0.19)	-14 (0.59)	-14 (0.24)
BASOPHIL %	-8.7 (0.72)	-3 (0.9)	13 (0.61)	-16 (0.55)	0.7 (0.95)

5-4. Discussion

5-4-1. Recapitulation of aims

This experiment had two main aims. These were, firstly, to assess the biological effect of a stressor treatment involving repeated exposure to varied acute stressors in adult laying hens and secondly, to investigate whether fractal behavioural complexity related to other measures of stress.

5-4-2. Did the stressor treatment really stress the birds?

Over S-1, body weight decreased in the stress group but not in the control group. The stress group did, however, recover with an increase in weight over S-2. This recovery of body weight in the stress group would seem to represent an adaptation to the stress treatment. A similar profile, of initial weight loss then recovery, has been found during repeated (daily) restraint in rats (Papaioannou et al. 2002). Over S-1, treated birds ate less than the controls, although the difference was not quite significant. Stressed birds ate significantly less than controls during S-2. A decrease in food intake and resulting weight loss is commonly seen in animal stress studies (Broom & Johnson 1993; Puvadolpirod & Thaxton 2000a). Food intake was measured for both test and companion birds, so the intake of the companion, which did not experience the full treatment, may mask any larger changes in the test birds' intake. Curiously, food intake significantly increased in both groups in S-2 compared to S-1[1]. There was no significant difference in egg production at any time point, although a couple of pens in the stress group showed large decreases in production. However, the weight of eggs laid by stress group birds decreased over the stress periods and in the following days. That this effect lasted beyond the stress periods is a small indication that the birds in the stress group may not have completely habituated to the stress treatment.

In the stress group, the heterophil to lymphocyte (H/L) ratio increased over the two stress periods, while in the control group it declined. However, at no point was there any difference between the two groups. The fact that the heterophils and lymphocytes and the ratio between them are significantly consistent for individual

[1] One bird that died during the experiment was later diagnosed as suffering from a coccidiosis infection. It could be that there was a general coccidiosis infection in the group at this time, which suppressed intake in all birds during S-1, but was overcome by the time of S-2.

birds across the four samples in the control group but not in the stress group could be taken as some evidence of stress in the stress group. Generally speaking, the values for the H/L ratios are high compared to previous work (Maxwell 1993). Most previous studies of H/L ratios have been undertaken in broilers or younger laying hens, so higher values could be normal for laying birds of this age. It is equally possible however that the values could reflect a high degree of stress in the whole population of birds for reasons unconnected with the experiment.

There was no difference, in corticosterone concentration between control and test birds for samples one or three. However, for sample two the control birds were found to have a higher corticosterone concentration than the test birds. This difference cannot be explained by sampling times; there was no correlation between sampling time and corticosterone concentration and no difference in sampling time between the treatments. It could be that the higher values in the control birds reflect an acute response to the handling or more generally to the commotion in the room during sampling. As part of the stressor treatment, stress group birds were handled a lot more than controls and may have habituated to handling. However, corticosterone concentration was not found to increase over the course of sampling the whole room. The increased values could also reflect genuine differences in baseline measures. A recent study that replicated the original CMS protocol similarly found that control rats had higher corticosterone levels than treated rats two weeks following the end of the treatment (Murison & Hansen 2001). Two possible explanations for this finding were suggested. The first explanation is that the repeated activation of the HPA axis caused an alteration in glucocorticoid feedback mechanisms, creating a state of hypocortisolism similar to that which occurs in humans with post traumatic stress disorder (PTSD) or chronic fatigue (Heim et al. 2000). Hypocortisolism has also been found in some animal models of chronic stress. Conversely, the second explanation, which seems to be favoured by the authors, is that the procedures were so mild that they were not stressful and indeed may have provided enrichment to the test animals. However, the presumption that enrichment would actively decrease glucocorticoid levels is not always valid. De Jong and colleagues (2000c) housed pigs in enriched and impoverished conditions and found that enrichment actually increased cortisol levels during the daytime active period. Although it could be doubted whether the protocols used here or by Murison and Hansen are severe enough to cause a PTSD type state the

point that repeated activation of the stress response can alter HPA function is important to keep in mind. However, the data do not necessarily support a suppression of corticosterone in the stress group, rather it looks more like an increase in the control group. Measurement of corticosterone concentration as an indicator of long-term welfare has produced many contradictory results. Such inconsistencies suggest that basal corticosterone may be a poor measure of chronic stress and long-term welfare (Rushen 1991).

In summary, the results of the experiment allow few unequivocal statements to be made. There were some alterations in the stress group that suggest some stress was imposed for part of the experiment. The time course of any stress and subsequent habituation is unknown.

5-4-3. Does behavioural complexity correlate with stress status?

There was no difference between stress and control groups, in the Detrended Fluctuation Analysis (DFA) alpha value for vigilance or activity, at any time point. However, the other measures used did not give an indication of a clear or consistent stress state in the stressor treatment group. Although the treatment groups did not differ in their H/L ratio, the variation in H/L ratio seen for all birds did allow the relationship between fractal behavioural complexity and stress to be tested. The H/L ratio is a well established measure of stress in chickens (Maxwell 1993). It therefore seemed reasonable to use the H/L ratio as the objective measure of stress against which to test DFA. One problem with this approach is the time lag between the behavioural observations and the blood sampling. Observations were made over the course of three days with the blood sample being taken on the fourth day. This means that behaviour was observed one, two or three days before the blood sample. The H/L ratio could alter dramatically in this time. However, such change would only act to mask any underlying relationship between the immune status of the animal and its behaviour. It would certainly not make a false positive more likely. The results for the comparison show that there is a highly significant relationship between the $A\alpha$ and the H/L ratio, as well as the individual percentage of heterophils, lymphocytes and monocytes in one of the four observations (observation three, following the end of S-2). The negative relationship between the $A\alpha$ and the H/L ratio indicates that as the H/L ratio increases (indicating greater stress) the pattern of activity becomes less structured and more

complex, the opposite of what would be predicted based on previous work (Alados et al. 1996; Alados & Weber 1999; Alados & Huffman 2000). The fact that the relationship is only seen in one of the observation/samples does raise a question over whether it is a reliable result or not, but it is a result that deserves further study.

It is possible that the behavioural observations may have missed the times of peak stress. Since each set of behavioural observations took three days, birds observed on the third day may have shown less of a change than those on the first day after S-1 or S-2. There does appear to have been a process of stress induction and then habituation (based on the body weight results) so it is possible that stress levels peaked at some point during S-1. However, since the primary interest was in chronic stress, making observations after the stressor application rather than during it was justified.

5-4-4. Other potential models of increased allostatic load in poultry

There are few well-validated models of chronic stress in poultry that could have been implemented with the resources available. A replicable and easily applied model of chronic stress in poultry would be extremely valuable, particularly when trying to identify behavioural correlates of chronic stress. Current behavioural assessments of different poultry housing systems focus mostly on the ability of birds to express their full behavioural repertoire. However, behavioural assessments of this sort can be confounded by the fact that the form of the environment dictates what behaviours an animal can show (Haskell et al. 1996). So observed differences do not necessarily indicate anything about the welfare of the animal. For instance, behaviour in an enriched environment will be different to behaviour in an impoverished one because the former gives the animal a larger variety of possibilities. With this in mind, to adequately test behavioural indicators of stress requires that stress can be induced in an environment within which controls can also be housed.

Artificially increasing allostatic load through exogenous activation of the HPA axis might provide a useful model of chronic stress, with which to assess the utility of fractal analysis. Continuous administration of ACTH through osmotic pumps for seven days has been used to model a reliable and demonstrable physiological stress in broiler chicks (Puvadolpirod & Thaxton 2000abcde). Behaviour was not observed during this study however, and it would be interesting to see how behaviour alters under such chronic elevation of corticosterone. Jones and colleagues (1988) showed that chronic

elevation of circulating corticosterone increased duration of TI in laying hens. Other potentially useful models of external stress might be social stress (e.g. Gross & Siegel 1981) or thermal stress (e.g. Bollengier-Lee et al. 1998), both of which could be applied in a constant environment. Various genetic selection models could also have been used. For instance, Satterlee and Johnson (1988) selected lines of Japanese quail on the basis of adrenocortical response to crush cage restraint. These lines of birds have subsequently been found to differ in their degree of developmental instability in a way that suggests that the line selected for a magnified acute stress response suffers from chronic stress (Satterlee et al. 2000). The magnified responses of these birds appear to be non-specific so they show increased stress reactivity to numerous stressors and are generally more fearful. This increased reactivity will place these birds under a greatly increased allostatic load compared to those selected for a low response, even in the same environmental conditions. This would make them a good model to assess behavioural indicators of stress such as fractal analysis.

5-5. Conclusion

The stressor regime used in this experiment did not cause a consistent stress state in the birds used. However, the alterations that were seen suggest that the initial series of acute stressors may have been stressful but that the birds adapted to the treatment. It does not appear, on the basis of this experiment, that behavioural complexity is a parameter that alters under chronic stress in poultry. However, it is debatable how good a test of the initial hypothesis this experiment provided. The decision to attempt a novel stress treatment was, with the benefit of hindsight, a mistake since the main aim of the work was to test fractal analysis. Any future attempt to test the validity and utility of fractal analysis should concentrate on simpler and reliable stress treatments, physiological intervention or genetically selected lines of birds.

Chapter Five Appendix

Table A5-1: Ethogram definitions, based on references in the literature

	Definition
States	
Stand head up	Bird is upright with sternum clear off ground, stationary on one or both legs. Head is above horizontal midline of body, back of head higher than line of back.
Stand head down	Bird is upright with sternum clear off ground, stationary on one or both legs. Head is below horizontal midline of body, back of head below line of back.
Stand preen	Bird is upright with sternum clear off ground, stationary on one or both legs. Bird directs attention with beak (pecking, stroking, combing or nibbling) towards body and feathers.
Walk head up	Bird takes at least two consecutive steps with head up.
Walk head down	Bird takes at least two consecutive steps with head down.
Sit head up	Bird's body resting on ground, with head up.
Sit head down	Bird's body resting on ground, with head down.
Sit preen	Bird's body resting on ground, preening.
Dustbathe	Bird engages in dustbathing behaviour, kicking litter onto body and wiggling body about in dust. Feathers ruffled.
Events	
Wing flap	Quick repeated movement of wings. Bird may or may not be moving.
Wing stretch	Bilateral or unilateral, upward and sideways extension of wing(s).
Test peck[2]	Test birds directs peck towards companion.
Companion peck[2]	Companion directs peck towards test bird.
Drink	Bird pecks at water drinker.
Body shake	Feathers raised and body shakes[3].
Head shake	Head is moved rapidly from side-to-side.
Ground scratch	Bird scratches in sawdust making backward strokes with leg: typically body moves down and bird moves forward then back.
Test feather peck[4]	Test bird directs a peck at the feather of the companion. Includes pecking and pulling feathers.
Companion feather peck[4]	Companion bird directs a peck at the feather of the test. Includes pecking and pulling feathers.
Tail shake[4]	Bird shakes tail from side to side without moving rest of body.

[1] Kruijt 1964; Black & Hughes 1974; Wood-Gush 1989
[2] In experiment one these categories included all forms of pecking
[3] In experiment one body shake included tail shaking.
[4] In Experiment Two the definitions in the shaded part of the table were added.

Table A5-2: Mean body weight (grams) of test birds in the stress and control groups at each of the four weighing points

	Stress	Control	Statistics	
Time point[1]	Mean (S.D.) Range	Mean (S.D.) Range	Test Statistic	P-value
W1	1920.7 (198.4) (1646-2226)	1890.2 (271.6) (1547-2271)	t=0.28 (df=17)	0.78
W2	1840.2 (164.4) (1664-2120)	1907.7 (218.3) (1643-2191)	t=-0.77 (df=17)	0.45
W3	1898.2 (172.9) (1640-2134)	1889.6 (224.3) (1516-2193)	t=0.09 (df=16)	0.93
W4	1912.9 (171.5) (1664-2192)	1934 (203.1) (1662-2256)	t=-0.24 (df=16)	0.81

[1] Refer to Figure 5-2 for timing of W1-W4.

Table A5-3: Percentage egg production[1] averaged over five-day batches

	Stress	Control	Statistics	
Period[2]	Mean (S.D.) Median Range	Mean (S.D.) Median Range	Test Statistic[3]	P-value
B1	78 (30.8) 90 (20-110)	80 (19.4) 90 (50-100)	W=100	P=1
B2	73 (23.1) 75 (40-100)	71.1 (16.2) 70 (40-90)	t=0.2	P=0.84
B3	72 (27.8) 85 (20-100)	73.3 (15.8) 70 (50-100)	W=105.5	P=0.68
S-1A	59 (32.5) 60 (0-100)	65.6 (18.8) 60 (40-100)	t=-0.53	P=0.6
S-1B	54.4 (28.3) 50 (0-90)	71 (21.5) 70 (40-100)	t=-1.41	P=0.18
M	61.1 (34.8) 50 (0-110)	73.3 (23.5) 80 (40-100)	t=-0.87	P=0.4
S-2A	63 (29.5) 60 (30-100	77.8 (26.8) 90 (30-110)	W=88	P=0.34
S-2B	76 (24.1) 80 (30-110)	73.8 (26.1) 80 (30-100)	t=0.19	P=0.85
A1	68 (26.6) 70 (30-100)	73.7 (29.2) 85 (20-100)	t=-0.44	P=0.67
A2	70 (23.1) 75 (40-100)	83.8 (18.5) 90 (40-100)	W=81.5	P=0.25
A3	72 (25.5) 71.7 (37-100)	76.3 (14.8) 76.7 (53-97)	t=-0.42	P=0.68

[1] Egg production is expressed as a percentage where 100% would be equal to every birds laying an egg every day.

[2] Refer to Figure 5-2 for details of the batch periods

[3] Two-sample T-test or Mann-Whitney test

Table A5-4: Egg weight (grams) averaged over five-day batches

	Stress	Control	Statistics	
Period[1]	Mean (S.D.) Range	Mean (S.D.) Range	Test Statistic[2]	P-value
B1	64.9 (4.8) (54.7-71.4)	65.2 (3.36) (61.1-71)	t=-0.19 df=17	P=0.85
B2	63.2 (3.54) (57.4-68.3)	65.3 (3.45) (61-70.9)	t=-1.31 df=17	P=0.21
B3	63.8 (4.4) (56.1-68.3)	65 (3.4) (59.8-70.3)	t=-0.68 df=17	P=0.47
S-1A	63.5 (3.8) (55.3-68.7)	64(4.2) (58.8-70.5)	t=-0.28 df=15	P=0.78
S-1B	60.7 (2.53) (57.8-65.4)	64.8 (3.68) (58.8-71)	t=-0.2.66 df=15	P=0.018/0.18
M	63.8 (2.85) (60.5-68.8)	65.1 (3.24) (60-70.4)	t=-0.96 df=16	P=0.35
S-2A	62.8 (2.7) (56.5-65.3) Median =63.7	66.2 (3.29) (62.1- 72) Median =66.3	W=76.5 df=16	P=0.06/0.6
S-2B	63.1 (2.34) (58.4-66.7)	66.6 (4.54) (60-71.2)	t=-2.11 df=16	P=0.051/0.51
A1	62.6 (4.6) (52.5-67.1)	67.5 (3.46) (62.9-72.5)	t=-2.49 df=16	P=0.024/0.24
A2	64.4 (2.9) (59.5-67.8)	66.9 (3.35) (62-71.2)	t=-1.73 df=16	P=0.1

[1] Refer to Figure 5-2 for details of the batch periods

[2] Two-sample T-test or Mann-Whitney test

Table A5-5: Eggshell breaking strength and related parameters

Measure	Group	Pre-stress	Post-stress	Difference (One-sample t-test)
Load at Limit (N)	Stress (n=8)	7.5	8.3	t=0.72, P=0.49
	Control (n=9)	8.1	8.4	t=0.26, P=0.8
	Statistics	t=-0.71, P=0.49	t=-0.06, P=0.95	
Work to Limit (J)	Stress (n=8)	0.013	0.013	t=0, P=1
	Control (n=9)	0.014	0.014	t=0.07, P=0.95
	Statistics	t=-1.59, P=0.13	t=-1.14, P=0.27	
Maximum load (N)	Stress (n=8)	35.7	35.0	t=0.04, P=0.97
	Control (n=9)	36.8	39	t=0.7, P=0.51
	Statistics	t=-0.54, P=0.6	t=-1.2, P=0.25	
Deflection at Maximum load (mm)	Stress (n=8)	0.37	0.37	t=0.68, P=0.52
	Control (n=9)	0.39	0.4	t=0.6, P=0.57
	Statistics	t=-1.25, P=0.23	t=-1.68, P=0.11	
Work to Maximum load (J)	Stress (n=8)	0.006	0.006	t=0.32, P=0.76
	Control (n=9)	0.006	0.007	t=0.97, P=0.36
	Statistics	t=-0.85, P=0.4	t=-1.34. P=0.2	
Stiffness (N/m)	Stress (n=8)	136270	142889	t=1.04, P=0.33
	Control (n=9)	141076	149771	t=1, P=0.35
	Statistics	t=-0.87, P=0.39	t=-0.65, P=0.52	
Width (mm)	Stress (n=8)	43.2	43.9	t=2.15, P=0.069
	Control (n=9)	44.1	44.1	t=-0.04, P=0.97
	Statistics	t=-2.24, P=0.04/0.08	t=-0.26, P=0.8	
Length (mm)	Stress (n=8)	58.7	60.0	t=1.79, P=0.12
	Control (n=9)	59.1	58.7	t=-0.61, P=0.56
	Statistics	t=-0.44, P=0.67	t=1.27, P=0.22	
Shell Thickness (mm)	Stress (n=8)	0.40	0.41	t=0.62, P=0.56,
	Control (n=9)	0.41	0.41	t=0.54, P=0.61
	Statistics	t=-0.28, P=0.78	t=-0.49, P=0.63	

Table A5-6: Statistics for total time spent vigilant, frequency of vigilant behaviours and mean duration of vigilant behaviours for each of the four behavioural observations in each of the two treatment groups

Observation	Treatment	Total time vigilant (seconds) Mean (S.D.) Range	Frequency of vigilant behaviours Mean (S.D.) Range	Mean duration of vigilance (seconds)[2] Mean (S.D.) Range
One	Stress	1300 (533) (702-2183)	160.1 (70.5) (66-272)	10.7 (Med.=7.1) (8.9) 3.9-31.9 (IQ=4.6-14.9)
	Control	1453 (502) (526-2035)	174.8 (48.6) (100-242)	9.1 (Med.=8.3) (4.8) 2.2-20.3 (IQ=7.2-10.0)
	Statistics	t=-0.64 P=0.53	t=-0.52 P=0.61	W=96 P=0.78
Two	Stress	1418 (553) (729-2274)	159.2 (73.3) (86-331)	10.5 (6.4) 3.1-22.7
	Control	1307 (435) (553-2200)	158 (40.8) (90-223)	8.9 (3.8) 3.1-15.3
	Statistics	t=0.48 P=0.64	t=0.04 P=0.97	t=0.68 P=0.51
Three	Stress	1442 (484) (915-2277)	184.2 (46.3) (138-273)	8.2 (Med.=7.7) (3.3) 4.3-14.0 (IQ=5.5-10)
	Control	1399 (456) (869-2419)	163.6 (73.9) (8-244)	45.1 (Med.=7.2) (108.5) 4.9-313.6 (IQ=6.3-7.8)
	Statistics	t=0.14[1] P=0.89	t=0.72 P=0.48	W=99 P=0.76
Four	Stress	1439 (675) (660-2903)	182.5 (123.6) (19-425)	26.7 (Med.=6.3) (44.8) 3.1-123.2(IQ=4.6-30.7)
	Control	1102 (407) (445-1763)	185.3 (27.3) (143-2222)	6.0 (Med.=5.5) (3.1) 2.3-12.2 (IQ=4.6-6.8)
	Statistics	t=1.17 P=0.26	t=-0.06 P=0.95	W=97.5 P=0.49

[1] Log-ten transformation used (back transformed means presented)
[2] Where data could not be transformed to normality, medians (med.) and Inter-quartile (IQ) range are given in addition to means and ranges.

Table A5-7: Statistics for total time spent in activity, frequency of active behaviours and mean duration of active behaviours for each of the four behavioural observations in each of the two treatment groups

Observation	Treatment	Total Time Active (seconds) Mean (S.D.) Range	Frequency of Active Behaviours Mean (S.D.) Range	Mean duration of activity (seconds) Mean (S.D.) Range
One	Stress n=10	240.1 (277.5) (39-956.8)	94.3 (94.1) (25-327)	2.1 (0.34) (1.6-2.8)
	Control n=9	214.1 (203.8) (20.3-578.8)	87.6 (79.8) (11-260)	2.1 (0.37) (1.8-2.9)
		t=0.11[1] P=0.91	t=0.11 P=0.91	t=-0.03 P=0.98
Two	Stress N=10	244 (531) 14-1744)	90 (185.9) 8-614)	2.3 (0.75) (1.6-4.3)
	Control n=9	154.6 (177.7) (34.1-601.7)	58.9 (52.9) (16-187)	2.1 (0.55) (1.2-2.8)
		t=-0.6[1] P=0.55	t=-0.72[1] P=0.48	t=0.68[1] P=0.51
Three	Stress n=10	248.0 (291.8) (38.4- 1019.8)	95.7 (102.9) (18- 360)	2.1 (0.48) (1.5- 2.9)
	Control n=8	102.4 (74.1) (32.2-236.9)	49.2 (35) (14-99)	2.0 (0.33) (1.5-2.4)
		t=1.53[1] P=0.14	t=1.23[1] P=0.24	t=0.66 P=0.52
Four	Stress n=10	301 (542) (16-1806)	127.6 (210.2) (5-696)	2.2 (0.4) (1.7-2.9)
	Control n=7	105 (63.8) (43.6-209.4)	44 (24.1) (17-81)	2.2 (0.3) (1.8-2.6)
		t=0.6[1] P=0.56	t=0.58[1] P=0.57	t=-0.1 P=0.92

[1] Log-ten transformation used (back transformed means presented)

Table A5-8: Duration (seconds: medians and inter-quartile range) of each individual behavioural state

Observation	Treatment	BEHAVIOURAL STATE					
		Stand head up	Stand head down	Stand Preen	Sit head up	Sit head down	Sit preen
One	Stress n=10	992 (611-1223)	1258 (743-186)	165 (117-639)	0 (0-11.7)	0 (0-63.7)	0 (0-1)
	Control n=9	1173 (720-1349)	1210 (986-1427)	298.7 (29.2-450.3)	0.8 (0-188.7)	10.4 (0-51.2)	0 (0-60.6)
	Statistics	W=91, P=0.49	W=101, P=0.97	W=103, P=0.84	W=84, P=0.21	W=87, P=0.31	W=92, P=0.54
Two	Stress n=10	902 (759-1529)	964 (437-1732)	372.2 (47.2-582.9)	44 (0-98)	84.2 (0-323.7)	4.2 (0-24.3)
	Control n=9	1060 (698-1345)	1346 (982-1652)	342.8 (79.3-406)	0 (0-67.9)	0 (0-57)	0 (0-10.7)
	Statistics	W=96, P=0.78	W=87, P=0.31	W=105, P=0.71	W=112, P=0.35	W=114, P=0.27	W=114, P=0.27
Three	Stress n=10	907 (691-1406)	1209 (810-1821)	179.3 (17.8-429.3)	99.6 (0-141.5)	45.8 (0-158.5)	4 (0-36.3)
	Control n=9	969 (744-1252)	776 (681-1402)	327.8 (46.7-626.7)	120 (25-343.70)	58.7 (0-210.5)	111.8 (0-248.1)
	Statistics	W=95, P=1	W=104, P=0.45	W=87, P=0.51	W=85, P=0.40	W=95, P=0.1	W=79.5, P=0.18
Four	Stress n=10	879 (665-1195)	1267 (245-1962)	55.1 (10-245.60)	0 (0-302.5)	0 (0-290.4)	0 (0-25.7)
	Control n=9	949 (602-1009)	1118 (574-1997)	266 (71-723)	17.4 (0-228.8)	0 (0-322)	0 (0-70.5)
	Statistics	W=91, P=0.96	W=85, P=0.66	W=75, P=0.16	W=88, P=0.88	W=87, P=0.81	W=93, P=0.81

Table A5-8 continued

Observation	Treatment	BEHAVIOURAL STATE			
		Walk head up	Walk head down	Dust bathe	Preen total
One	Stress n=10	109.2 (37.9-303)	16.07 (8.1-40.33)	0 (0-0)	204 (117-639)
	Control n=9	82.9 (56-335.9)	33.45 (8.31-50.71)	0 (0-0)	344.7 (29.2-455.6)
	Statistics	W=102, P=0.9	W=92, P=0.54		W=101, P=0.97
Two	Stress n=10	39 (20-137)	6.6 (4.5-31.8)	0 (0-0)	402.2 (47.2-596.4)
	Control n=9	70.3 (43.8-154.7)	21.1 (10.14-36.3)	0 (0-101.1)	364.3 (79.3-461.8)
	Statistics	W=89, P=0.39	W=90, P=0.44		W=108, P=0.54
Three	Stress n=10	104.7 (61.2-309.1)	11.6 (5-44.9)	0 (0-13.4)	223.9 (24.9-450.5)
	Control n=9	62.7 (33.3-130.9)	18.56 (2.97-39.9)	0 (0-15.6)	569 (56-875)
	Statistics	W=110, P=0.20	W=95, P=1	W=94, P=0.96	W=81, P=0.23
Four	Stress n=10	61.9 (34.9-204.2)	21.6 (9.7-128)	0 (0-0)	64.3 (10-249.30)
	Control n=9	67.6 (24.5-120.3)	21.8 (12.2-46.29)	0 (0-0)	485 (71-723)
	Statistics	W=94, P=0.73	W=89, P=0.96		W=74, P=0.13

Table A5-9: Median (inter-quartile range) frequency of behavioural events for each of the four observations and each of the two treatments

		BEHAVIOURAL EVENT					
Observation	Treatment	Flap	Stretch	Test	Buddy	Drink	Body Shake
One	Stress n=10	2 (0-3)	0 (0-1.25)	1.5 (1-8.75)	2 (0-3.25)	5 (0- 21.5)	1 (0.75-2)
	Control n=9	1 (0.5- 3.5)	1(0.5- 1.5)	5 (0-9)	1 (0- 9)	13.7- 29.5)	1 (0-1)
	Statistics	W=101, P=0.97	W=82.5, P=0.13	W=99, P=0.93	W=98.5, P=0.93	W=79, P=0.09/ 0.36	W=111, P=0.35
Two	Stress n=10	1.5 (0-3)	0.5 (0-1)	1.5 (0- 6.75)	0 (0-1)	4.5 (1- 14.75)	1 (0-1)
	Control n=9	1 (0-2.5)	1 (0-1.5)	1 (0-2)	1 (0.5- 5.5)	15 (4.5-64.5)	1 (0.5- 3)
	Statistics	W=101.5, P=0.93	W=90, P=0.4	W=106, P=0.65	W=78, P=0.06/ 0.24	W=83, P=0.18	W=85.5, P=0.22
Three	Stress n=10	1 (0.75- 2)	0.5 (0- 1.25)	1.5 (0- 99.2)	0 (0- 7.75)	17.5 (3.75- 32.25)	1 (1- 2.25)
	Control n=9	0.5 (0- 1.75)	1.5 (1-2)	2.5 (0- 10.25)	0 (0-1.75)	32 (13-73)	1 (0.25- 1)
	Statistics	W=104, P=0.43	W=77, P=0.1	W=102, P=0.55	W=98, P=0.8	W=82, P=0.27	W=109.5, P=0.17
Four	Stress n=10	0 (0-1)	0 (0-2)	5 (0.75- 14.75)	0.5 (0-11)	6 (3-24.75)	1 (0-1)
	Control n=9	1 (0-3)	2 (0-2)	2 (0-15)	1 (0-3)	22 (5- 40)	1 (1-1)
	Statistics	W=76, P=0.14	W=83, P=0.49	W=93, P=0.81	W=87, P=0.8	W=79, P=0.3	W=84, P=0.53

Table A5-9 continued

Observation	Treatment	BEHAVIOURAL EVENT					
		Head shake	Scratch	Tail shake	Buddy feather peck	Test feather peck	Comfort[1]
One	Stress n=10	17.5 (7.5- 24.3)	32 (14.3-82.7)	0 (0-1)	0 (0- 1.25)	1.5 (0-7.25)	4 (1-7)
	Control n=9	7 (4- 15)	17 (4.5-32)	1 (0-2.5)	0 (0-12)	1 (0-8)	4 (3-8)
	Statistics	W=122, P=0.08 CP=0.32	W=117.5, P=0.16	W=86, P=0.24	W=94, P=0.62	W=99, P=0.97	W=95.5, P=0.74
Two	Stress n=10	19.5 (8.75- 40.5)	6 (1-23.5)	1 (0-1.25)	0 (0- 1.5)	2.5 (0-18)	3.5 (2.75- 5.25)
	Control n=9	8 (4.5- 19)	11 (0-37.5)	1 (0-1)	0 (0- 7.5)	0 (0-2)	3 (2-10.5)
	Statistics	W=120, P=0.11	W=99.5, P=1	W=103, P=0.82	W=93, P=0.49	W=120, P=0.1	W=97.5, P=0.87
Three	Stress n=10	8 (4- 20)	11.5 (5.25-55.5)	0 (0-1)	0 (0- 2.5)	0 (0-4.75)	4 (3.5-4.5)
	Control n=9	8 (1.25- 17.7)	3 (1.25-26.5)	0 (0-0.75)	0 (0-2.25)	0 (0-1.5)	3.5 (1.5- 4.75)
	Statistics	W=107.5, P=0.28	W=107.5, P=0.29	W=101, P=0.55	W=100, P=0.63	W=102, P=0.49	W=102.5, P=0.51
Four	Stress n=10	6.5 (3.25- 20.75)	56.5 (0- 113.5)	0 (0- 0.25)	0 (0-4.25)	0.5 (0-36.2)	2 (0.75- 4)
	Control n=9	8 (3- 24)	25 (5-127)	0 (0-2)	0 (0-2)	0 (0-0)	4 (2-6)
	Statistics	W=86, P=0.73	W=86.5, P=0.77	W=80, P=0.25	W=89, P=0.95	W=103, P=0.15	W=73.5, P=0.11

[1] **Since most of the behavioural events occur infrequently a composite named 'comfort' was calculated which comprises flapping, stretching, body shaking and tail shaking.**

Table A5-10: Leucocyte profiles[1] (mean (S.D.))for each treatment group, over the four repeated blood samples

Sample	Treatment group	Heterophil %	Lymphocyte %	H/L ratio	Monocyte %	Eosinophil %	Basophil %
One	Stress n=10	31 (11.98)	61.1 (11.97)	0.57 (0.35)	3.5 (1.78)	1.7 (1.3)	2.7 (1.9)
	Control n=9	35.67 (12.87)	55.22 (13.65)	0.72 (0.42)	3.2 (1.6)	2 (1.6)	3.9 (1.7)
	Statistics	t=-0.82 P=0.42	t=1 P=0.33	t=-0.95 P=0.35	t=0,36 P=0.72	t=-0.46 P=0.65	t=-1.44 P=0.17
Two	Stress n=10	36.6 (7.53)	54.5 (9.23)	0.71 (0.24)	3.5 (2.7)	2.6 (2.9)	2.8 (1.9)
	Control n=9	34.67 (9.71)	54.89 (10.41)	0.68 (0.32)	3.6 (1.5)	2.4 (1.4)	4.4 (2.9)
	Statistics	t=0.49 P=0.63	t=-0.09 P=0.93	t=-0.18 P=0.86	t=-0.05 P=0.96	W=94.5 P=0.68	t=-1.46 P=0.16
Three	Stress n=10	35.7 (9.82)	53.6 (11.29)	0.73 (0.34)	5.1 (2.4)	2.7 (1.9)	2.95 (1.6)
	Control n=8	30.9 (10.65)	59.56 (11.48)	0.58 (0.34)	4.3 (2)	0.8 (1)	4.4 (2.8)
	Statistics	t=0.99 P=0.34	t=-1.11 P=0.29	t=0.95 P=0.36	W=111 P=0.16	W=118.5 P=0.03/ 0.12	t=-1.37 P=0.19
Four	Stress n=10	27.7 (10.3)	64.7 (11.24)	0.47 (0.25)	3.3 (0.95)	1.7 (1.4)	2.6 (2.2)
	Control n=8	30.63 (8.26)	62.38 (8.75)	0.51 (0.19)	3.6 (1.6)	0.5 (0.76)	2.88 (1.36)
	Statistics	t=-0.65 P=0.52	t=0.48 P=0.64	t=-0.44 P=0.67	t=-0.54 P=0.6	W=115 P=0.07/ 0.28	t=-0.31 P=0.76

[1]One hundred cells were counted on each slide. Slides were randomly selected, observer was blind to treatment.

Table A5-11: Relationship (correlation percentage (p-value)) between leucocyte values and the total time vigilance, the frequency of vigilant behaviour and the mean bout duration of vigilance

		H/L ratio	Heterophil %	Lymphocyte %	Monocyte %	Eosinophil %	Basophil %
Observation One	Time	23 (0.35)	17 (0.49)	-22 (0.38)	-3 (0.9)	9 (0.72)	32 (0.18)
	Freq.	-3 (0.9)	3 (0.89)	0.9 (0.97)	-15 (0.54)	-15 (0.55)	-4 (0.88)
	Mean	22 (0.36)	13 (0.61)	-20 (0.42)	34 (0.16)	6 (0.8)	18 (0.46)
Observation Two	Time	8 (0.73)	10 (0.69)	-14 (0.58)	3 (0.9)	-4 (0.87)	19 (0.43)
	Freq.	11 (0.65)	13 (0.6)	-11 (0.67)	-13 (0.6)	-12 (0.64)	18 (0.47)
	Mean	-3 (0.92)	-3 (0.91)	3 (0.92)	12 (0.62)	-2 (0.93)	-9 (0.71)
Observation Three	Time	28 (0.26)	30 (0.23)	-30 (0.23)	29 (0.25)	5 (0.85)	-17 (0.5)
	Freq.	-46 (0.06/0.24)	-38 (0.12)	41 (0.1)	-62 (0.007/ 0.028)	-3 (0.91)	30 (0.23)
	Mean	46 (0.06)	41 (0.09)	29 (0.24)	49 (0.04)	2 (0.93)	-39 (0.11)
Observation Four	Time	34 (0.18)	29 (0.27)	-39 (0.13)	12 (0.64)	55 (0.02/ 0.08)	17 (0.53)
	Freq.	-32 (0.2)	-26 (0.32)	21 (0.43)	-3 (0.92)	24 (0.36)	2 (0.93)
	Mean	55 (0.02/0.08)	48 (0.05/0.2)	-48 (0.05/0.2)	3 (0.92)	37 (0.14)	-10 (0.7)

Table A5-12: Relationship (correlation percentage (p-value) between leucocyte values and the total time active, the frequency of active behaviour and the mean bout duration of activity

		H/L ratio	Heterophil %	Lymphocyte %	Monocyte %	Eosinophil %	Basophil %
Observation One	Time	-9 (0.73)	-11 (0.66)	11 (0.67)	-34 (0.15)	25 (0.31)	12 (0.63)
	Freq.	-5 (0.85)	-8 (0.74)	6 (0.8)	-26 (0.28)	28 (0.25)	15 (0.55)
	Mean	0.3 (0.99)	0.2 (0.99)	9 (0.73)	-58 (0.01/0.04)	2.7 (0.91)	-8 (0.73)
Observation Two	Time	31 (0.2)	34 (0.15)	-28 (0.25)	-14 (0.58)	-14 (0.57)	15 (0.53)
	Freq.	29 (0.22)	33 (0.17)	-26 (0.28)	-14 (0.57)	-13 (0.58)	13 (0.6)
	Mean	34 (0.16)	40 (0.09/0.36)	-36 (0.13)	10 (0.69)	-2 (0.95)	-3 (0.91)
Observation Three	Time	-10 (0.68)	4 (0.89)	9 (0.73)	-40 (0.1)	-11 (0.67)	-12 (0.64)
	Freq.	-10 (0 .7)	4 (0.87)	7 (0.78)	-38 (0.12)	-11 (0.66)	-9 (0.71)
	Mean	-31 (0.21)	-20 (0.42)	29 (0.24)	-47 (0.05/0.2)	-33 (0.19)	16 (0.52)
Observation Four	Time	-20 (0.43)	-20 (0.44)	12 (0.66)	0.3 (0.99)	53 (0.03)	-0.1 (99.7)
	Freq.	-22 (0.41)	-21 (0.43)	13 (0.63)	0.2 (0.99)	53 (0.03/0.12)	-3 (0.91)
	Mean	12 (0.66)	6 (0.83)	-14 (0.6)	38 (0.13)	18 (0.5)	6 (0.81)

Table A5-13: Relationship (correlation percentage (p-value) between the weight change over certain time periods and DFA alpha values in subsequent observations

Correlation between			
Weight change	Observation	$V\alpha$	$A\alpha$
W2 – W1 (S-1)	Two	-5 (0.83)	1 (0.69)
W3 – W2 (S-2)	Three	-5 (0.89)	29 (0.25)
W3 – W1 (S-1 & S-2)	Three	-5 (0.94)	36 (0.15)
W4 – W3 (Recovery)	Four	-38 (0.13)	-28 (0.27)

Chapter Six

The responses of growing pigs to a chronic stress treatment involving social and environmental stressors

Abstract

The aim of this experiment was to investigate the measurement of pig behaviour using the fractal analysis technique of Detrended Fluctuation Analysis (DFA) in the context of the behavioural and physiological effects of a stressor treatment involving repeated social and environmental disturbances.

Ten juvenile male pigs were exposed to up to five aggressive interactions with a larger aggressive pig. In addition, following the interactions, these pigs were housed for 42 hours in sensory contact with the larger pig and its group of companions. For the second 21 hours of this period they was also exposed to either wetting or removal of bedding, or inescapable airflow. The impact of this treatment on the test pigs, compared to littermate controls, was assessed through pre- and post-treatment measures of body weight, home pen behaviour and circadian salivary cortisol profile, as well as behaviour in three post-treatment open field tests.

There was a significant interaction between treatment and time in body weight gain over the stressor period ($P < 0.001$). Over the stressor period absolute body weight gain was significantly lower in test pigs than in control pigs ($P = 0.005$). Prior to the stress treatment there was no difference between test and controls pigs in their mean cortisol concentration over the full 24 hours ($P = 0.19$). However, after the stress treatment, test pigs had a higher salivary cortisol concentration than control pigs ($P = 0.019$). Test pigs increased the number of scans spent lateral lying in the home pen in the post-stress observation compared to the pre-stress observation ($P = 0.015$). Consequently, they spent less time ventral lying than control pigs in the second observation ($P = 0.01$). The DFA of postural activity in the home pen identified differences between the test and control groups, with test pigs having a more structured pattern of activity than controls in the second observation ($P = 0.036$) and a tendency towards a more structured pattern in the first observation ($P = 0.075$). Over all the pigs, the mean salivary cortisol concentration was significantly correlated ($r= 0.62$, $P=0.003$) with the fractal structure of postural activity following the stress treatment. The three repeated open field tests did not reveal any major differences between the two groups.

The results suggest that the stressor treatment did create a mild chronic stress, as indicated by the hypercortisolaemia and lower weight gain in the test pigs, relative to controls. The results of the behavioural analysis show that fractal

techniques, such as DFA, can be applied to pig behaviour and that they can reveal extra novel information about the structure of an individual's behavioural organisation.

6-1. Introduction

Numerous animal models of depression have been developed with the aim of aiding drug discovery and elucidating the biological mechanisms underlying depression and the effects of depression on the individual (Willner 1984, 1997a; Geyer & Markou 1995; D'haenen & Andrews 2000). These models range from those that only model transient symptoms of the human disorder to those where a long-term state similar to depression appears to be induced. Models of depression can be genetic, pharmacological or behavioural (D'haenen & Andrews 2000). Behavioural models involve exposing animals to a variety of stressor treatments. These stressor treatments, used to induce depression in animals in the laboratory setting, often have parallels in animal agriculture.

Two of the commonest behavioural models of depression are the social defeat model (e.g. Koolhaas et al. 1990; Meerlo et al. 1996abc, 1999) and the Chronic Mild Stress (CMS) model (Willner et al. 1987; Willner 1997a). Both of these involve treatments that may occur in intensive pig farming. Social defeat may be a common occurrence for some pigs in industry conditions, where the mixing of unfamiliar animals is common. Following mixing, defeated animals cannot avoid exposure to dominant animals and harassment of some pigs by aggressive individuals may occur (D'Eath 2002). Mixed pigs may show a decreased growth rate (Rundgren & Löfquist 1989; Stookey & Gonyou 1994; D'Eath 2002) and may become more susceptible to disease (de Groot et al. 2001). Fighting amongst pigs is therefore viewed as a potentially stressful experience. Indeed, at the time of aggression pigs show a classic stress response, with increased heart rate (de Jong et al. 2000a), catecholamine release (Otten et al. 1999, de Groot et al. 2001) and cortisol release (Otten et al. 1999, de Groot et al. 2001). The CMS model also has parallels in the day-to-day treatment of many farm animals, involving as it does, an irregular sequence of stressors coupled with environmental alterations. Ladewig (2000) has suggested that such intermittent application of different stressors is more relevant to the experience of farm animals than other stressor regimes. The CMS itself involves a

social stress component, which has been shown to make a major contribution to the overall effect of the treatment (Muscat & Willner 1992).

In this experiment, the stressor treatments were based around such models of social defeat and CMS. One of the most pertinent variants of the social defeat model is the social defeat plus sensory contact model. In the rodent version of this model (Kudryavtseva & Avgustinovich 1998; Keeney & Hogg 1999) the test animal is exposed to a different individual on a daily basis. Following the defeat from that individual the test animal is moved to another cage and kept in sensory contact with a new male. In the tree shrew version (Fuchs et al. 1996; Fuchs & Flügge 2002) an intruder male is introduced into the home cage of another (socially experienced) male. The two animals interact until a clear dominant-subordinate relationship is formed whereupon they are separated by a barrier allowing olfactory, visual and auditory but not physical contact. This barrier is removed for one hour every day to create a chronic stress state, as indicated by a hypercortisolism and increased urinary noradrenalin levels, in the subordinate male (Fuchs & Flügge 2002). The model also causes a general reduction in locomotory behaviour and reductions in feeding and drinking, resulting in a loss of body weight.

A sensory contact model of social defeat may be a more realistic simulation of the normal experiences of pigs, where losers must remain in the same pen as their defeaters. The treatment application used here, while different to both the rodent and tree-shrew models retains the principle of exposing a smaller animal to a larger aggressive animal and then subsequently housing them in sensory contact. In addition to this social stress treatment, pigs were exposed to environmental stressors in the form of substrate removal or wetting, or unavoidable airflow, whilst in the sensory contact set-up. The aim of this was to expose the pigs to dual environmental and social stressors, with the aim of creating a chronic stress, such as that induced by the CMS model.

The behavioural and physiological measures taken to assess the effect of the stressor treatment were based on previous work in pigs and also on measures commonly used in animal models of depression. The circadian rhythm of salivary cortisol was measured once before and once after the stressor treatment. In human depression and in animal models of depression the most common physiological indicator of depression is hypercortisolism (Steckler et al. 1999; Newport &

Nemeroff 2001; Parker et al. 2003). Home pen behaviour was assessed over 24 hours, once before and once after the stressor treatment. An alteration in the circadian rhythm in various biological variables, including glucocorticoid profiles and activity patterns, has also been commonly found in animal models of depression (Gorka et al. 1996; Meerlo et al. 1996a, 1999; Bunney & Bunney 2000). Behavioural markers of depression include reductions in feeding, social, and exploratory behaviours with a corresponding general increase in inactivity, known as psychomotor retardation in the human literature (Harlow & Suomi 1974; Blanchard et al. 2001b). Additionally, following the stressor treatment, behavioural responses to an open field and novel object test were observed. Reductions in locomotory behaviour and exploration in an open field have been suggested as indicators of depression in animal models (Meerlo et al. 1996; Kudryavtseva & Avgustinovich 1998; D'Aquila et al. 2000; Sumner et al. 2002). The open field test was repeated to assess whether the treatment groups differed in how they habituated to the repetition of the test.

The hypothesis being tested was that a stressor treatment involving social and environmental stress would alter the behavior and physiology of growing pigs in a way suggesting the presence of chronic stress or depression. One of the potential behavioural alterations was a reduction in behavioural complexity, as measured by Detrended Fluctuation Analysis (DFA). DFA had previously been used to identify subtle alteration in the vigilance behaviour of chickens following an acute stressor (Chapter Four) and has also identified changes in the behaviour of minnows exposed to lead pollution (Alados & Weber 1999) and chimpanzee suffering from ill health (Alados & Huffman 2000). Both these latter studies found a decrease in behavioural complexity in animals exposed to forms of chronic challenge. The hypothesis in the present work was that a chronic stress state, resulting from the stressor treatment described, would cause a decrease in the behavioural complexity of growing pigs. The identification of such an alteration might aid future assessments of chronic stress states in pigs.

6-2. Animals, materials and methods

6-2-1. Animals

All experimental work detailed here was carried out at SAC's Easter Howgate pig unit, following ethical approval by the animal experiments committee at SAC.

Sows of various parities were moved to the farrowing house around five days prior to giving birth. Sows were housed in farrowing crates and were provided with small amounts of straw and wood shavings. The 11 litters of Large White x Landrace piglets used in the experiment were born between 29/9/01 and 19/10/01. From birth onwards piglets had access to a heated creep area and the farrowing area was provisioned daily with fresh straw. At three or four days of age all piglets were given an iron injection. Male piglets were not castrated and teeth were not clipped. At approximately four weeks of age piglets were weaned. At this point, piglets were weighed, ear tagged and sexed. For the period following weaning and before the experiment began all pigs were kept in their litter groups, in large pens with straw bedding and *ad libitum* access to feed and water. During this time lights were dimmed but not completely switched off between 4pm and 8am.

6-2-2. Outline of the method

The experimental methods involved several stages. Firstly, the various pigs to be used in the experiment were selected from the available litters. A group of aggressive pigs were required to administer social defeats to a group of test animals. These aggressive pigs were selected on the basis of their attack latency, as described below in 6-2-3. The experimental pigs (tests, controls and companions) were selected, as described in 6-2-4. Following selection all these animals were moved to new housing, as described in 6-2-5. The experimental schedule is shown in Figure 6-1. Once underway, the experiment involved removing test pigs from their group and repeatedly exposing them to one of the aggressive resident pigs and other stressors, as described in section 6-2-6. Various behavioural and physiological measures were taken before and after the stressor treatment, as described in 6-2-8.

6-2-3. Selection of aggressive pigs

Prior to the start of the stress period, pigs from the eldest five litters were screened for attack latency in a resident-intruder test (e.g. Erhard & Mendl 1997). The heavier pigs in these litters were tested, as residents, against intruder pigs from younger litters. Residents and intruders were selected from both sexes as Erhard and Mendl (1997) found no apparent effect of resident or intruder sex on the resulting attack latency. (Subsequent to completion of the experiment, D'Eath and Pickup (2002) report some minor sex effects in the attack latency test: females attacked quicker and more often in the first of two tests and males where more likely to be attacked in the second of two tests). Although it would have been preferable to always use naïve intruders (because the previous experience of the intruder could potentially alter the outcome; Nelson & Chiavegatto 2000), due to the low number of pigs available for use as intruders, some individuals were used up to four times.

Two pairs of attack latency tests were carried out. The tests were done on consecutive days, two to three weeks before the start of the stress treatment, when the residents were 9½ -10½ weeks old. For each resident (n=25) a different intruder was used in each repetition of the test. For the test, a subsection of the resident's home pen was created. The resident pig was then placed on its own in the front section of the pen and the intruder was removed from its home pen and introduced into the resident's pen. Previous work (Erhard & Mendl 1997) suggested that the intruder pig should be lighter than the resident pig but not so light that the experimental pig does not attack it. A weight difference of approximately two-thirds was suggested as optimal and resident and intruders were matched as close to this as possible (Tables A6-1 to A6-4). Over all the tests the intruder was on average 69.6% (S.D. = 7.1) of the residents weight.

The time until the first clear nose of the intruder by the resident was recorded. If no nose occurred in the first five minutes then the test was stopped. The time from the first nose to an attack by the resident (or occasionally the intruder, in which case the test was stopped) was recorded as the attack latency. An attack was defined as a sudden lunge by the attacking pig followed by rapid or persistent biting. Immediately following an attack the two pigs were separated and the intruder was removed from the pen. If no attack occurred in the five minutes following the first nose then the test was stopped. The test was also stopped, and the intruder

removed, on the few occasions that the resident mounted the intruder more than ten times or if the intruder showed ten upright-escape attempts (e.g. the intruder reared up and placed its front legs on the side of the pen).

The results from the first pair of attack latency tests (Tables A6-1 & A6-2) were not clear cut enough for reliably aggressive residents to be picked. Due to this the pigs were kept in the same housing and another pair of tests were carried out when the residents were 14½ -15½ weeks of age, six to eight days prior to the start of the stressor treatments. The second pair of tests was carried out in the same manner as the first except that the maximum duration following the first nose was increased to ten minutes and one of the 25 pigs was omitted (due to extreme repeated mounting in the first test). In the second pair of tests the pigs were generally seen to be more aggressive (Tables A6-3 & A6-4) and it was possible to select 14 (four groups of three littermates and one group of two littermates) pigs for use as aggressive residents. Selection was on the basis of short attack latency and consistency of attacking. In the case of the pair of pigs, one of the pair was not seen to be very aggressive but its single littermate was highly and reliably aggressive. The 14 chosen pigs were moved to the resident pens five days prior to the first defeat.

6-2-4. Selection of experimental pigs

Ten triplets of littermates consisting of two males and one female were selected, at approximately eight weeks of age, from the remaining six litters. In addition to ear tags, experimental pigs were marked (either with spray: Ritchey SuperSprayline Stockmarker, or with marker pen: ZebraCon Ltd, McKie Extra Bold) on their back for quick identification during the experiment and on camera. The triplets were designated as 'test', 'control' (both male) and 'companion' (female). The decision as to which of the two males was the control and which was the test was made on the basis of size, as a crude surrogate measure for social status. The pairs were chosen where possible to have a large weight difference and the smaller pig was always chosen as the test pig. (See examination of this decision in section 6-4-3). A larger companion female was also used where possible.

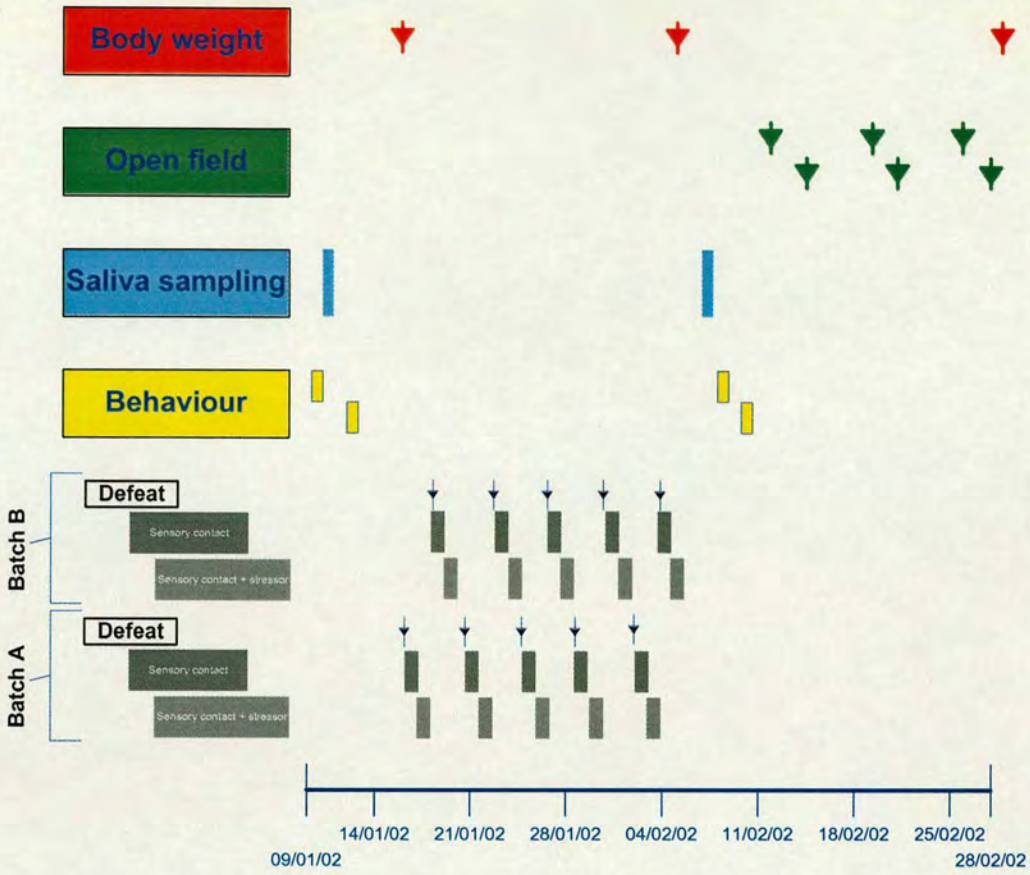


Figure 6-1: Experimental schedule
The post-stress home pen observations and the open-field testing were staggered in two batches to match the defeat batches

6-2-5. Housing of residents and experimental pigs

Following selection, the triplets of experimental animals were moved to the experimental building (Fig. 6-2) and were housed in pens (2.85m x 1.85m) with straw bedding. Five larger (5.75m x 1.85m) pens, in a different room, housed the pairs or triplets of aggressive residents selected in the attack latency test (Fig. 6-3). Experimental and resident pigs remained in these pens for the duration of the experiment. All pigs had *ad libitum* access to food and water throughout. Every pen was mucked out and provisioned with fresh straw daily between 8am and 10am. The main lighting in the experimental building was switched off between 6pm and 7am. Temperature in the experimental rooms varied between 15 and 18° C.

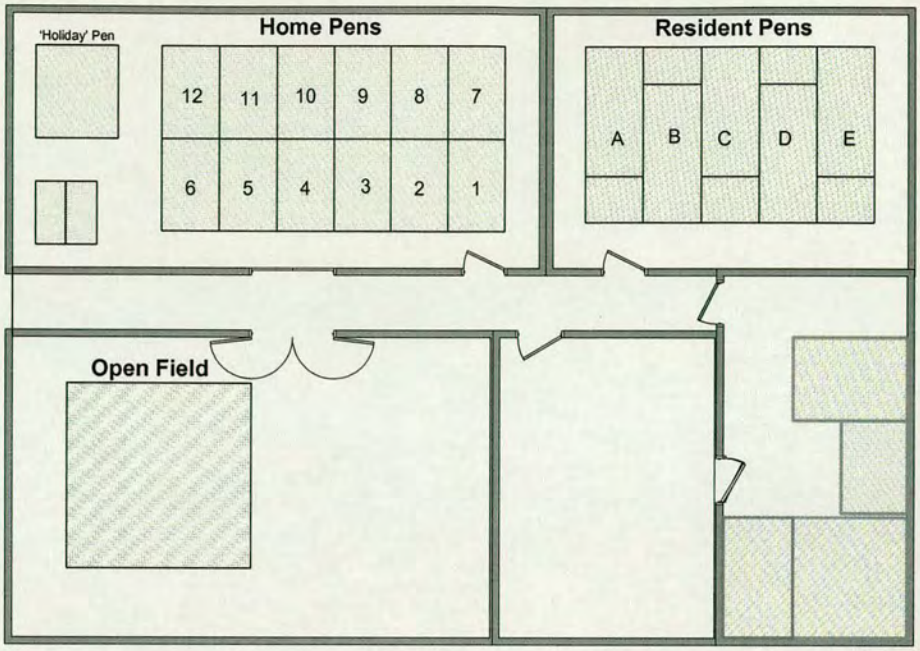


Figure 6-2: Layout of pens and rooms in the experimental building

Figure 6-3: Resident pens from above
From the top of the picture down, pens A to D are visible.

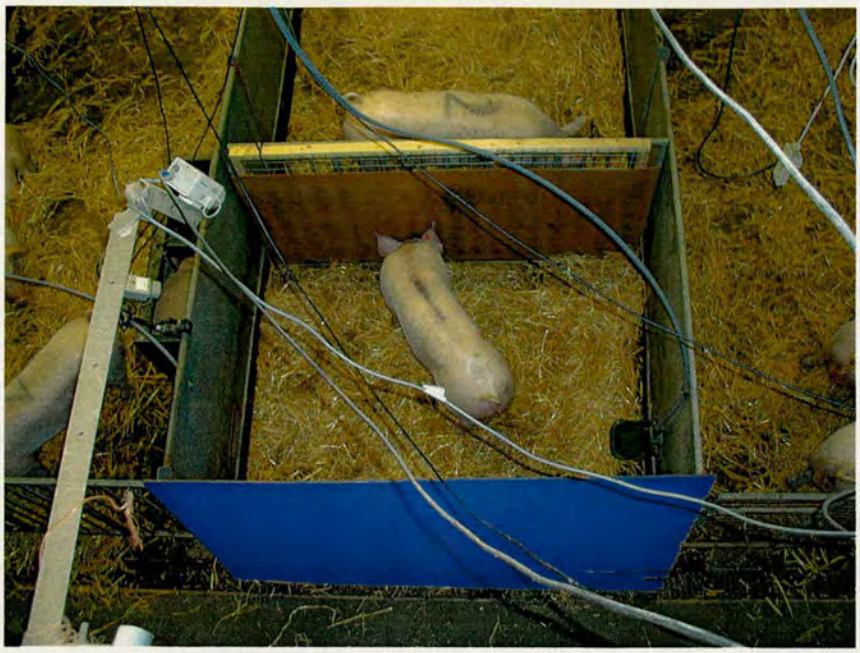


Figure 6-4: Enclosed end of resident pen, prior to aggressive interaction

The photograph shows one resident pig enclosed in a subsection of its home pen prior to the arrival of the test pig.

6-2-6. Treatment of the test pigs

The test pigs were exposed to a stressor treatment involving repeated aggressive social interactions, extended sensory contact with larger unfamiliar pigs and additional environmental stressors. The following process was repeated five times for nine out of the ten test pigs. One pig received only four repetitions because of ill health at the start of the experiment.

Immediately prior to the start of the interaction all the test pigs and all the residents were saliva sampled in their home pen. Following this, the first resident pig was moved into an enclosed section (1.85m x 1.44m; Fig. 6-4) at one end of its home pen. During the interaction with the test pig the gate separating them from the pen mates of the resident was solid. The test pig was then removed from its home pen and moved into the pen with the resident. The strategy for pairing test pigs and residents was based on:

1) The weight difference between resident and intruder (66% is optimal; see Tables A6-5 to A6-9 for details of actual weights).

2) Using residents with a reliable attack record.

3) Not repeating any pairings of test pigs and residents.

4) No repeat test pig visits to the same pen (even with a different resident).

The interaction between the two pigs was deemed to have started at the point when the attack latency test would normally finish, i.e. the first attack by either pig and was stopped on the basis of several different possible criteria. The principal criterion was a time limit, following the last clear bite aimed at the resident by the intruder, of ten minutes in the first interaction and five minutes in subsequent ones. This was based on Rushen and Pajor's (1987) finding that the cessation of attacking by one individual pig in a dyadic encounter represents a turning point in the interaction. They found that the form of fighting between two unacquainted pigs (and putatively their underlying motivational state) evolves over the course of an interaction. Although they note that there may not be any clear-cut transition from offence to defence in the losing pig they suggest that the point at which the losing pig stops biting is the clearest available indicator of this change. Following this point the majority of the losers' time is spent in defensive postures.

If both pigs continued to fight the interaction was stopped 20 minutes from the first attack. If neither pig showed any aggression the test was stopped 30 minutes from the time the test pig entered the pen. The interaction was also stopped if the resident pig showed excessive mounting, defined as 20 mounts, or earlier if mounts were long lasting or caused damage to the test pig's back. In addition, the interaction was stopped on ethical grounds if the aggression shown by either pig was considered extreme, e.g. if either pig was suffering excessive damage to its skin (particularly if one pig directed bites repeatedly towards the same area of skin).

Following the interaction the resident was returned to its companions. The test pig remained in the small subsection, separated from the residents by a barred gate preventing direct aggressive physical contact but still allowing visual and some degree of physical contact (i.e. the pigs remain in sensory contact; Fig. 6-5a). Both test pig and resident were saliva sampled five minutes following the end of the interaction. The test pig remained in this pen for approximately 42 hours from the start of the encounter, during which time it had *ad libitum* access to feed and water. For the first 21 hours the test pig was not exposed to any additional stressors, while in the second 21 hour period it was exposed to one out of the following: removal of

bedding, wetting of bedding or unavoidable airflow. These stressors were only ever applied singly. Following the first and fourth defeats substrate removal was used (Fig. 6-5b). Following the second defeat substrate wetting was used. Following the third and fifth defeats the test pigs were exposed to increased airflow over their section of the pen, using a large fan (Oscillating desk fan: PlazaAir Model AM-16, 40cm) positioned at the front of the pen (Fig. 6-6). At the end of the 42-hour period the test pig was returned to its home pen.

The test pigs were tested in two alternating batches of five animals. For each batch of five, the aggressive interactions took place on the same day. Whilst one batch was being housed in the sensory contact situation the pigs in the other batch were in their home pens. Consequently, there was a four-day gap between each aggressive interaction. There was a gap of six hours between consecutive test pigs being housed in the front area of a particular defeat pen (i.e. between a batch A pig being returned to its home pen and the next, batch B, test pig interacting with a resident). This was to allow the resident a period of access to this area before the next interaction.

a b

Figure 6-5: Test pigs housed in sensory contact with residents
a: In initial sensory contact
b: In second period of sensory contact (in this case during substrate removal).

Figure 6-6: The set-up to apply unavoidable airflow to the test pig

6-2-7. Treatment of the control pigs

On each occasion when the test pig was away from the home pen (for the interaction with the aggressive resident and subsequent sensory-contact housing) the control and companion pigs were removed from the home pen and taken to another pen, designated the 'holiday' pen (see Fig. 6-2), for 30 minutes. This process was intended to habituate control pigs to being away from the home pen and also gave them a similar level of handling experience to test pigs.

6-2-8. Assessment of treatment effects

6-2-8-1. General

Pre-treatment measurements of body weight, home pen behaviour and the circadian rhythm of salivary cortisol concentration were made (see Fig. 6-1). These measurements were repeated following the stress period. In addition, following the stress period three repeated open field tests were carried out. Where possible post-treatment measures were staggered so that they occurred at the same time since the last defeat for the two batches.

In addition to the measures described here, a sucrose preference test was used before and after the stressor treatment to allow an assessment of any stress-induced anhedonia. These data were analysed by Still (2002) in her B.Tech.Ag. thesis and will not be discussed further in this chapter.

6-2-8-2. Cortisol

Cortisol concentration in saliva was measured as an indication of HPA axis activity. Saliva samples were collected by allowing the sampled pig to chew on a large cotton bud (Millpledge) until sufficient saliva was deposited on the cotton. Typically this took around 30-60 seconds and sampling all 20 pigs took around 15-20 minutes. The cotton buds were then placed in 7ml S-Monovette tubes (Sarstedt) and spun in a centrifuge for five minutes at 3000 rpm and 4ºC. The saliva was then poured off into another tube and frozen. These samples were sent to an external laboratory for cortisol assay. Samples were assayed in duplicate and the minimum detectable cortisol concentration was 0.5 ng/ml.

On two occasions (five days before the stress treatment and four days after; see Fig. 6-1) a saliva sample was taken from test and control pigs every one and a half hours for a 24-hour period (16 samples in total from each animal) to assess circadian rhythmicity. Acute responses to the open field and aggressive interaction were also measured by taking a sample before and after these procedures. Both the test and resident pigs were sampled before and at five minutes following the end of the interaction. Pigs were also sampled before the open field test and ten minutes after (both in the home pen).

6-2-8-3. Home pen behaviour.

For the home pen observations two 24-hour video recordings of each home pen were made, with cameras (Panasonic WV-BP120, with Panasonic AG-TL300B Time-lapse video recorder) positioned above the front of each pen. The first observation was six days before the start of the stress treatment and the second was five days following the end of the stress treatment (see Fig. 6-1). Scan samples of test- and control-pig behaviour were made every minute using an ethogram (Table 6-1) divided into three hierarchical categories: a posture, a behaviour and a substrate.

The main lighting within the experimental room was switched off at night. However, lighting on a gantry above the pens was left on overnight to allow test and control pigs to be identified, on the video recordings, and their postures clearly recorded. The practice of starting to leave some lights on all night was started one week before the first observation and continued throughout the experiment.

Table 6-1: Ethogram for home pen observations

Category	Name	Definition
Posture		
	LATERAL LIE	Pig lies on side with pelvis and shoulder in contact with ground.
	VENTRAL LIE	Pig has body in contact with ground. Lying on front with front legs forward
	SIT	Pig has rear in contact with ground, body raised up on front legs.
	STAND	Pig is up on all four legs; entire torso is raised off ground.
	WALK	Pig is up on all four legs and moving at time of sample.
Behaviour		
	ALERT	Head up, ears are pricked; attention is paid to surrounding environment.
	IDLE/DOZE	Pig is not engaged in any interaction with the environment. Includes sleeping.
	CHEW	Pig chews substrate with visible jaw movements.
	NOSE	Nose in contact with substrate.
	ROOT	Pig has snout down in contact with substrate. Moves snout back and forth in substrate.
	SOCIAL	Social behaviour, e.g. 'play' fighting, head knocking.
Substrate		
	NONE	Pig's snout is not in contact with any substrate.
	STRAW	Pig has snout in contact with straw, either on ground or chewing.
	WALL	Pig has snout in contact with wall or any part of pen fixtures apart from feeder and drinker.
	PIG	Pig is either nosing other pig or engaged in social interaction.
	FEEDER	Head is down in feeder.
	DRINKER	Head is in drinker.
	PERSON	Snout in contact with person (only seen during brief time for mucking out).

6-2-8-4. Open field test plus novel object.

The control and test pigs were exposed to an open field test on three occasions (9, 16 & 23 days following the last return to the home pen; Fig. 6-1). The test involved taking the pigs one at a time to a large open field arena (3.75m x 3.75m, Fig. 6-2 and 6-7) in a different room and observing their behaviour in the arena for a total of ten minutes. The ten-minute period started with a five-minute period of observation in the empty open field. Then a novel object was introduced into the pen through a hole in one side of the arena (Fig. 6-8) and the final five minutes of observation were of the interaction with the novel object, as well as general behaviour. The floor of the pen was covered in straw taken from pens of other pigs to provide the arena with a pig odour. At one end of the open field a video camera (as for home pen observations) was suspended 2.8m of the round. One pig was tested from each pen in a randomly determined sequence. Whether the control or test pig was tested first was also decided randomly. Once the first pig from every pen had been tested, the second pig was then tested (in the same order). Behaviour in the open field was recorded onto video and analysed later using Keytime (Deag 1993), with an ethogram (Table 6-2) divided into postures and behaviour-substrate combinations. The number of defecations was also recorded, as was the latency to make contact with the novel object.

The room in which the open field was set up had Yorkshire boarding and wind sheets on two sides. The temperature in the open field arena was therefore lower than in the home pen room: day 1 = 8° C, day 2 = 3-5° C, day 3 = 6° C, day 4 = 3° C, day 5 = 3-5° C) and day 6 = 5° C.

Figure 6-7: Open field set-up
Note the square hole in the far left wall, through which the novel object was introduced.

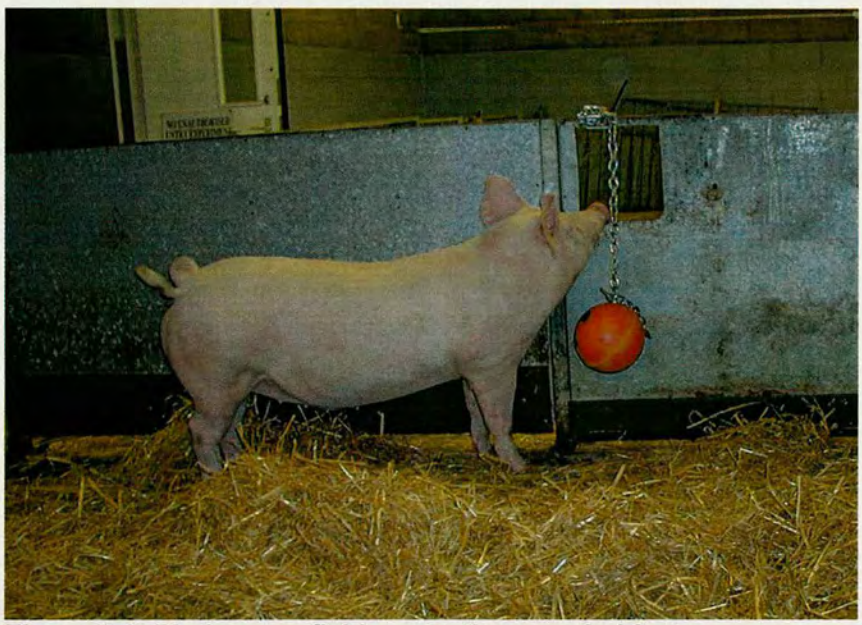


Figure 6-8: Pig in open field contacting novel object

The novel object was an orange plastic fishing float (64cm in circumference), hanging 30cm off the ground and attached by a metal chain to the top of one side of the pen.

Table 6-2: Ethogram for open field observations

Category	Name	Definition
Posture		
	Stand	Pig is up on all four legs; entire torso is raised off ground. Pig is motionless
	Walk	Pig moves forward or back, taking more than one clear step.
	Run	Pig moves quickly forward.
	Sit/Lie	Pig either sits with rear in contact with ground or fully lies down with body in contact with substrate
Behaviour + Substrate		
	Root-Straw	Pig has head down, snout in contact with substrate, rooting or chewing.
	Nose-Wall (+post holes)	Close nosing of wall. Also licking at postholes.
	Nose-Gate	Nosing at any point along the length of the gate to the pen.
	Nose-Novel object	Snout is in contact with the novel object
	Idle-None	Pig stands with head lowered. Pig is not in contact with any substrate.
	Alert-None	Pig stands still with head up and ears pricked.
	Chew-Novel object	Pig chews chain holding novel object in place
	Push-Gate	Pushing at exit to open field.
Events		
	Defecate	Bolus of faeces is deposited.
	Latency to touch object	Time from introduction of novel object to first physical contact by pig with snout.

6-2-9. Data Analysis

6-2-9-1. Body weight

The measurements of body weight were analysed using a repeated measures ANOVA (Genstat 2001), with treatment groups blocked by pen and the body weight prior to the first defeat used as a covariate.

6-2-9-2. Cortisol

For each pig a mean cortisol concentration was calculated from the 16 samples taken over 24hours. Paired t-tests were used to assess differences between test and control pigs before and after the stressor treatment. To compare values during the light and dark periods for each pig a mean cortisol value was calculated for periods with the light on (6.30pm, 7.30am to 4.30pm values) and light off (7pm to 6am values). Paired t-tests were used to test for differences between light and dark periods in test and control pigs, before and after the stress period.

For the aggressive interactions, only the cortisol samples from interaction number two were analysed.

6-2-9-3. Home pen behaviour

The number of scans recorded in the various categories was analysed. Paired t-tests were used to compare the numbers of scans recorded in each category between test and control groups. The sequences of behaviour were also analysed using the fractal analysis technique of Detrended Fluctuation Analysis (DFA). The DFA is the same as that applied in Chapters Four and Five. In this case, however, the cumulative behavioural score is created using the sequences of scan samples, so the resolution of the analysis is one minute rather than half a second. The complexity of the fluctuation between various combinations of behaviour (Table 6-3) was assessed.

Table 6-3: Categories of fluctuation used for DFA

Name	Fluctuation between
Postural Activity	Lateral lie + ventral lie + sit versus all other postures
Behavioural Activity	Idle-doze versus all other behaviours
Feeding	Feeder + drinker versus all other substrates
Interaction	None versus all other substrates
Straw	Straw versus all other substrates
Social	Social versus all other behaviours
Pig	Pig versus all other substrates
Lateral	Lateral lie versus all other postures

6-2-9-4. Open field behaviour

A repeated measures ANOVA (Genstat 2001) was used to assess treatment (between subject) effects, time (within subject) effects and interactions between the two for the major behavioural categories. Degrees of freedom were corrected (for autocorrelation within individuals) using the Greenhouse-Geisser epsilon value, prior to assigning P-values. For these categories and for more specific behavioural subsets differences between the treatments were assessed using paired t-tests. Although the animals were tested individually in the open field the fact that the pairs of test and control animals were littermates, of the same sex that had been housed for the majority of their life in the same conditions justified using a paired test, as the animals are clearly not independent.

6-2-9-5. Presentation

Unless otherwise stated data are presented as mean ± S.D.

6-3. Results

6-3-1. Attack latency tests

The results of the attack latency tests are presented in Tables A6-1 to A6-4. In the final attack latency test the pigs chosen as residents had a mean attack latency of 36.9 seconds. The distribution of attack latencies (over all four tests) (Fig. 6-9) is similar to those published in the literature (D'Eath & Burn 2002; Erhard & Mendl 1997). The total number of attacks increased over the four tests and there was an

apparent priming effect, indicated by a reduction in latency to attack, in the second test of each pair (Fig. 6-10).

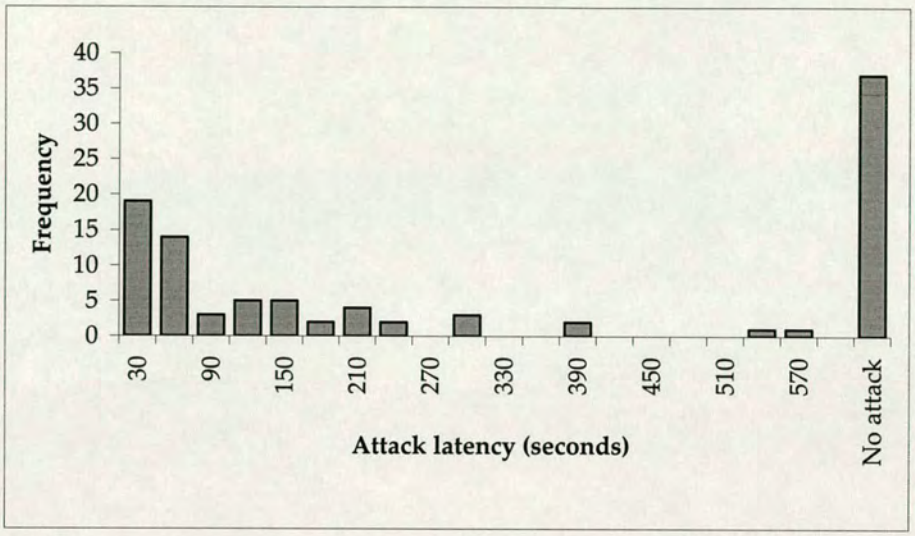


Figure 6-9: Frequency distribution of all attack latencies (n=98)

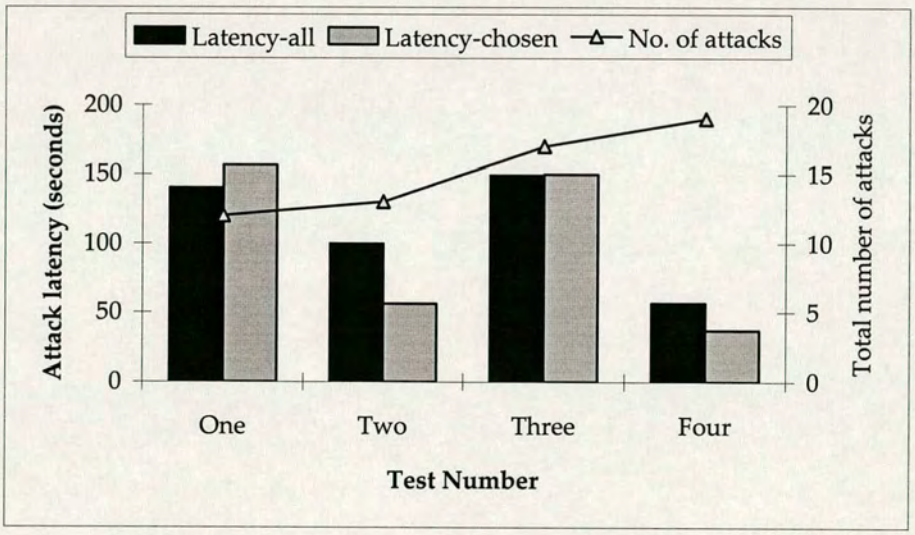


Figure 6-10: Total number of attacks and mean attack latency for all attacking pigs and for pigs subsequently chosen as aggressive residents, over the four attack latency tests

For the first and second tests: n=25. For the third and fourth tests: n=24.

6-3-2. Aggressive interactions

In many cases five clear defeats for each individual did not occur. Using the expression of submissive/escape behaviour by the intruder pig as a crude indicator of defeat, there was found to be a spectrum of intruder experience from no defeats to all defeats (Tables A6-5 to A6-9). Given this the results cannot be interpreted as the effects of a repeated social defeat. However, all test pigs were exposed to social stress and the rest of the treatment occurred as planned. In the first round of interactions eight out of the ten were considered to result in a defeat for the test pig. However, by the fifth round of interactions, only three out of the nine resulted in defeats.

Generally, when the test pigs were returned to their home pen they were accepted back by the other two pigs. However, on four occasions aggression (anything beyond mild interactions such as nosing, pushing, head knocking) by one or both of the control and companion pigs was directed towards the returning test pig. On these occasions a paper feed sack was thrown into the pen to distract the pigs from their aggression and this intervention was always successful in stopping the aggression.

6-3-3. Body weight

Immediately prior to the start of the stress treatment, control pigs had a mean weight of 60.6kg (S.D. = 6.9) and test pigs had a mean weight of 50.4kg (S.D. = 6.45). The difference between individual pairs ranged from 0 to 22kg. The resident animals had a mean weight of 71.4kg (S.D. = 6.2).

The change in body weight of both test and control groups is shown in Figure 6-11a. The repeated measures ANOVA revealed no significant effect for treatment ($F_{1,8}$=0.67, P=0.44). However, there was a significant interaction between treatment and time ($F_{3,54}$=16.69, P<0.001). The interaction between treatment and time is illustrated in Figure 6-11b showing the change in body weight in the two groups when the pre-treatment body weight difference is used as a covariate to calculate adjusted means. Not surprisingly, there was a significant effect of time on body weight ($F_{3,54}$=441.6, P<0.001). Over the period of the stress treatment the absolute weight gain was significantly greater in control animals (Control=23.95 ±3.4 Kg vs. Test=17.96 ±3.3 Kg: one-sample t-test, t=-3.74, P=0.005). There was no difference

between the two groups in the absolute weight gain during the post-stress period (Control=15.37Kg ±2.4 vs. Test=14.95Kg ±1.9: t=-0.4, P=0.7), however, test pigs showed a greater relative increase in weight (Control=18.36% ±3.3 vs. Test= 22.03% ±2.7: t=3.99, P=0.003).

Interestingly, over the stress period the aggressive resident animals gained significantly less weight than control animals (Control=23.95Kg ±3.4 vs. Resident=19.05Kg ±3.1: Two-sample t-test, t=-3.56, P=0.002) and showed a reduced relative weight gain compared to both control (Control=39.8% ±36.1 vs. Resident=26.8% ±3.8: t=-6.33, P<0.001) and test (Test=35.7% ±5.7 vs. Resident=26.8% ±3.8: t=-4.54, P<0.001) animals.

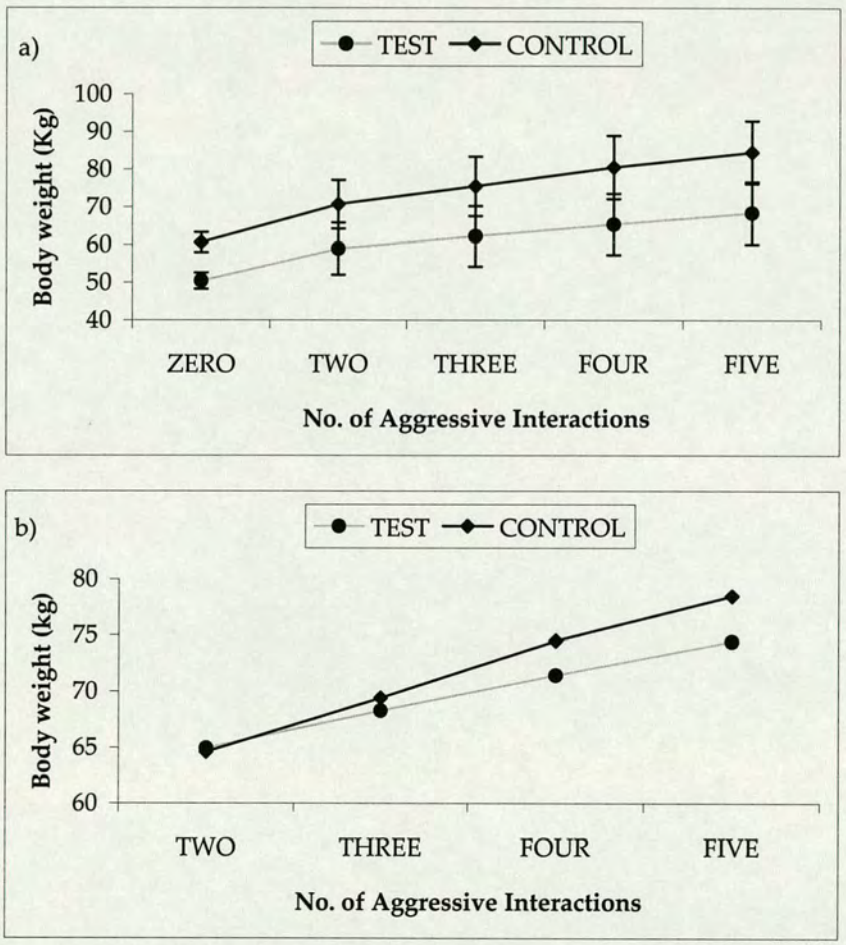


Figure 6-11: Change in body weight over the stressor period
a) original data, b) data adjusted for starting weight as a covariate.

6-3-4. Salivary cortisol concentration

Prior to the stressor period there was no difference between test and control pigs in their mean cortisol concentration (Test = 1.51ng/ml ±0.89, Control = 1.15ng/ml ±0.36: t=1.41, P=0.19). However, following the period of stressor exposure, test pigs had a higher salivary cortisol concentration than controls (Test = 1.45ng/ml ±0.42, Control = 1.05ng/ml ±0.23: t=2.84, P=0.019). The profiles of cortisol concentration over the 24hours period before and after the stressor period are shown in Figures 6-12 and 6-13.

Cortisol concentration was significantly greater during daylight than at night for test pigs before (Paired t-test, Log Ten transformation: t= -3.2, P=0.011) and after (Log Ten, t=-6.43, P<0.001) the stressor period and also for control pigs before (Log Ten, t=-2.61, P=0.028) and after (t=-5.74, P<0.001) the stressor period.

There was no difference in the salivary cortisol concentration between test and control pigs before or after any of the three open field tests (Table 6-4). In the second test, both test and control pigs showed a significant increase in cortisol concentration after the test compared to their levels before.

There was no difference between test and resident pigs in their salivary cortisol concentration prior to the second aggressive interaction (Medians: Test = 0.91ng/ml vs. Resident =0.79ng/ml, Mann-Whitney test, W=113, P=0.57). Over the course of the aggressive interaction the cortisol concentration significantly increased in the test pigs (One-Sample t-test: t=-2.94, P=0.017) but not in the residents (t=0.55, P=0.59). This meant that following the interaction test pigs had a significantly higher cortisol concentration than residents (Medians: Test = 2.39ng/ml vs. Resident =0.96ng/ml, Mann-Whitney test, W=146, P=0.002).

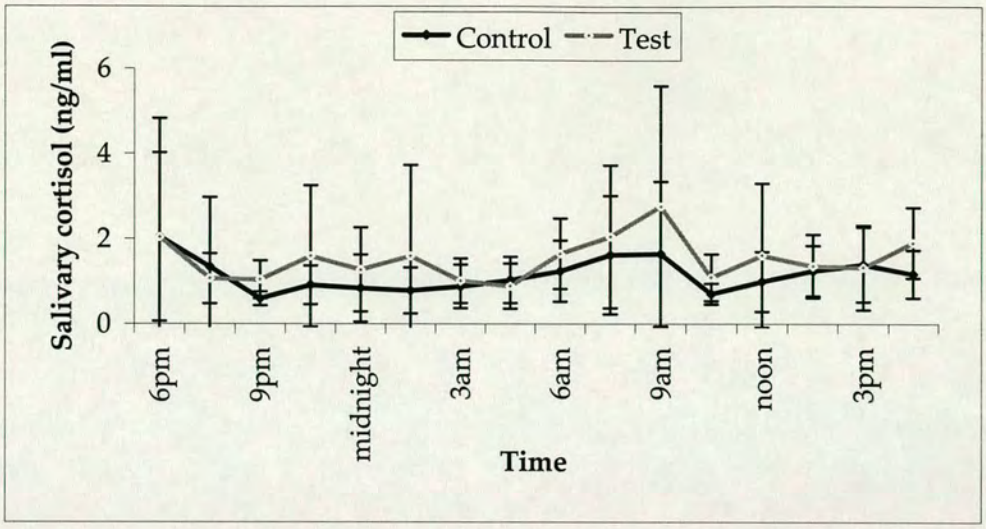


Figure 6-12: Salivary cortisol concentration (Mean ± S.D.) over 24hours, prior to stressor period, for both control and test pigs

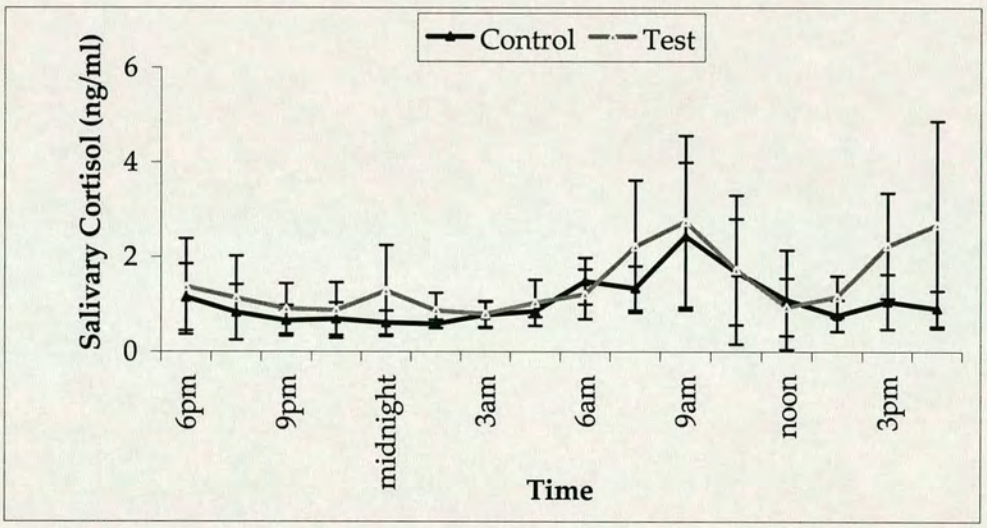


Figure 6-13: Salivary cortisol concentration (Mean ± S.D.) over 24hours, after the stressor period, for both control and test pigs

Table 6-4: Salivary cortisol concentrations before and after the three open field tests (ng/ml: Mean ± S.D.)

Test number	Before			After			Before vs. After	
	Control	Test	Test vs. Control	Control	Test	Test vs. Control	Control	Test
One[1]	1.65 (1.08)	2.26 (1.69)	t=1.23 P=0.26	1.9 (0.56)	2.56 (1.22)	t=1.38 P=0.23	t=-0.52 P=0.62	t=-0.59 P=0.58
Two	0.93 (0.86)	0.86 (0.75)	t=-0.64 P=0.54	1.27 (0.63)	1.73 (0.47)	t=1.72 P=0.12	t=-2.78 P=0.02	t=-2.94 P=0.016
Three	1.08 (0.71)	1.1 (0.72)	t=0.1 P=0.92	1.9 (1.5)	2.5 (2.1)	t=1.23 P=0.25	t=1.23 P=0.25	t=-1.87 P=0.094

[1] There were some missing samples in the first test, due to a problem with the supplier of the cotton buds. Sample sizes are therefore: Control before=9, Test before = 8, Control after = 7, Test after = 7.

6-3-5. Home pen behaviour - Standard analysis

Prior to the stressor treatment there were no differences between the two groups in the postures they adopted (Table 6-5). The only difference between the two groups following the stress treatment was in ventral lying; control pigs spent significantly longer ventral lying than test pigs. Between the pre-stress and post-stress observations test pigs tended to decrease the amount of time spent ventral lying and significantly increased the amount of time lateral lying. There was a trend towards test pigs spending more time walking than control pigs in the post-stress observation. Control pigs significantly decreased the amount of time walking in the post-stress observation compared to the pre-stress observation, while test pigs showed no difference.

In the behavioural categories (Table 6-6), there was a trend towards test pigs showing more rooting than control pigs following the stress treatment. Control pigs decreased the amount of time spent nosing and increased the amount of time idling/dozing in the second observation compared to the first. Following the stress treatment there were no differences between the two groups in the substrates they directed their attention towards (Table 6-7). Control pigs increased the amount of time they spent not directing contact to any substrate in the second observation. Both groups decreased the time spent in contact with straw in the second observation. Test pigs tended to direct more attention to the pen wall in the second observation, although the number of scans recorded in this category is low in both observations.

The pattern of behaviour over 24h was broadly similar for all categories (Fig. 6-14 – 6-17). The observations started at 5pm, so the first bin represents the last hour of light before the lights were put out at 6pm. Following this there is a decline in activity. Over the night there is some intermittent activity, until around 6am when the amount of activity increases. There is a distinct peak of activity around 8am. This corresponds to the time when the pens were being mucked out and having fresh straw added. Following this time there is a period of general inactivity around 10am to 11am, followed by another peak of activity around noon. The pigs are then intermediately active over the course of the afternoon. There was very little difference between the control and test pigs in the circadian pattern of any of the behavioural categories.

Table 6-5: Home pen observations: postures

	Before stress treatment					After stress treatment					
	Control		Test			Control		Test			
Posture	Total scans: Mean (S.D.)	%: Mean (S.D.)	Total scans: Mean (S.D.)	%: Mean (S.D.)	Test vs. Control	Total scans: Mean (S.D.)	%: Mean (S.D.)	Total scans: Mean (S.D.)	%: Mean (S.D.)	Test vs. Control	Before vs. After: Control / Test
Lateral lie	694.7 (175.5)	48.2 (12.2)	692.3 (143.5)	48.1 (10)	t=0.05 P=0.96	715.6 (136)	49.7 (9.4)	777.1 (123.5)	54 (8.6)	t=-1.84 P=0.1	t=-0.45 P=0.66 / t=-25.98 P=0.015
Ventral lie	510.3 (168.8)	35.4 (11.7)	487.7 (178)	33.9 (12.4)	t=0.44 P=0.67	505.6 (129.2)	35.1 (9)	415.4 (138.7)	28.8 (9.6)	t=3.26 P=0.01	t=0.11 P=0.92 / t=1.76 P=0.11
Sit	5.6 (5.27)	0.39 (0.37)	5.5 (6.62)	0.38 (0.46)	t=0.05 P=0.96	10.4 (14.93)	0.72 (1)	6.9 (6.9)	0.48 (0.48)	t=0.77 P=0.46	t=-1.35 P=0.21 / t=-1.35 P=0.21
Stand	202.5 (38.1)	14.1 (2.6)	227.8 (52.1)	15.8 (3.6)	t=-1.27 P=0.24	191.7 (43)	13.3 (3)	218.5 (35)	15.2 (2.4)	t=-1.73 P=0.12	t=1.3 P=0.23 / t=0.38 P=0.71
Walk	26.9 (8.6)	1.9 (0.6)	26.7 (11.79)	1.9 (0.82)	t=0.04 P=0.97	16.7 (10.59)	1.2 (0.7)	22.1 (6.87)	1.5 (0.5)	t=-1.84 P=0.099	t=2.83 P=0.02 / t=1.03 P=0.33

Table 6-6: Home pen observations: behaviours

	Before stress treatment					After stress treatment						
	Control		Test			Control		Test			Before vs. After	
Behaviour	Total scans: Mean (S.D.)	%: Mean (S.D.)	Total scans: Mean (S.D.)	%: Mean (S.D.)	Test vs. Control	Total scans: Mean (S.D.)	%: Mean (S.D.)	Total scans: Mean (S.D.)	%: Mean (S.D.)	Test vs. Control	Control	Test
Alert	56 (48)	3.9 (3.3)	32.1 (31.1)	2.2 (2.2)	t=1.68 P=0.13	33.8 (22.7)	2.3 (1.6)	23.7 (31.8)	1.6 (2.2)	t=1.38 P=0.2	t=1.69 P=0.13	t=2.12 P=0.063
Idle-doze	1072.6 (45.9)	74.5 (3.2)	1076.5 (74.2)	74.8 (5.2)	t=-0.14 P=0.89	1142.9 (58.8)	79.4 (4.1)	1126 (49.9)	78.2 (3.5)	t=0.82 P=0.44	t=-3.08 P=0.013	t=-1.64 P=0.14
Chew	18.5 (9.54)	1.3 (0.7)	19.6 (10.2)	1.4 (0.7)	t=-0.29 P=0.78	25.4 (11.6)	1.8 (0.8)	22.8 (12.2)	1.6 (0.8)	t=0.95 P=0.37	t=-1.46 P=0.18	t=-0.81 P=0.44
Nose	177.6 (25.6)	12.3 (1.8)	170.8 (51.7)	11.9 (3.6)	t=0.42 P=0.69	140.1 (29.7)	9.7 (2.1)	148 (35.2)	10.3 (2.4)	t=-0.66 P=0.53	t=3.02 P=0.014	t=1.01 P=0.34
Root	101.8 (34.3)	7.1 (2.4)	127.6 (45.3)	8.9 (3.1)	t=-1.61 P=0.14	85.1 (45.6)	5.9 (3.2)	106.1 (30.4)	7.4 (2.1)	t=-1.84 P=0.099	t=1.93 P=0.086	t=1.23 P=0.25
Social	13.5 (9.2)	0.9 (0.6)	13.4 (10.1)	0.9 (0.7)	t=0.05 P=0.96	12.7 (10.7)	0.9 (0.7)	13.4 (10.4)	0.9 (0.7)	t=-0.64 P=0.54	t=0.3 P=0.77	t=0 P=1

Table 6-7: Home pen observations: substrates

	Before stress treatment					After stress treatment						
	Control		Test			Control		Test			Before vs. After	
Substrate	Total scans: Mean (S.D.)	%: Mean (S.D.)	Total scans: Mean (S.D.)	%: Mean (S.D.)	Test vs. Control	Total scans: Mean (S.D.)	%: Mean (S.D.)	Total scans: Mean (S.D.)	%: Mean (S.D.)	Test vs. Control	Control	Test
None	1128.6 (40.8)	78.4 (2.8)	1108.6 (71.4)	77 (5)	t=0.9 P=0.39	1176.7 (49)	81.7 (3.4)	1149.7 (41.4)	79.8 (2.9)	t=1.27 P=0.24	t=-2.8 P=0.021	t=-1.36 P=0.21
Straw	198.3 (41.2)	13.8 (2.9)	225.3 (63.8)	15.6 (4.4)	t=-1.33 P=0.22	154.6 (44.4)	10.7 (3.1)	175.6 (32.2)	12.2 (2.2)	t=-1.23 P=0.25	t=2.73 P=0.023	t=2.37 P=0.042
Wall	7.1 (3.96)	0.5 (0.3)	4.5 (2.95)	0.3 (0.2)	t=2.35 P=0.043	5.9 (3.87)	0.4 (0.3)	8.2 (5.87)	0.6 (0.4)	t=-1.1 P=0.3	t=1.23 P=0.25	t=-2.2 P=0.055
Pig	27.3 (17.64)	1.9 (1.2)	21.2 (13.21)	1.5 (0.9)	t=0.91 P=0.39	22.7 (15.18)	1.6 (1.1)	23.1 (12.13)	1.6 (0.8)	t=-0.09 P=0.93	t=1.53 P=0.16	t=-0.52 P=0.62
Feeder	68.2 (15.54)	4.7 (1.1)	71.2 (27.25)	4.9 (1.9)	t=-0.3 P=0.77	68.3 (10.63)	4.7 (0.7)	71.8 (17.62)	5.0 (1.2)	t=-0.56 P=0.59	t=-0.02 P=0.99	t=-0.06 P=0.95
Drinker	9.9 (6.57)	0.7 (0.5)	8.9 (2.77)	0.6 (0.2)	t=0.45 P=0.66	11.4 (5.17)	0.8 (0.4)	11.4 (4.06)	0.8 (0.3)	t=0 P=1	t=-1.03 P=0.33	t=-1.76 P=0.11
Person	0.6 (0.7)	0.04 (0.05)	0.3 (0.48)	0.02 (0.03)	t=1.41 P=0.19	0.4 (0.52)	0.03 (0.04)	0.2 (0.42)	0.01 (0.03)	t=1.5 P=0.17	t=0.8 P=0.44	t=0.43 P=0.68

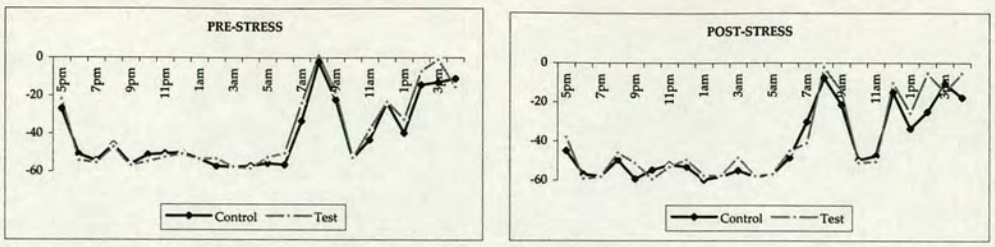


Figure 6-14: Circadian pattern of postural activity, pre- and post-stress[1]

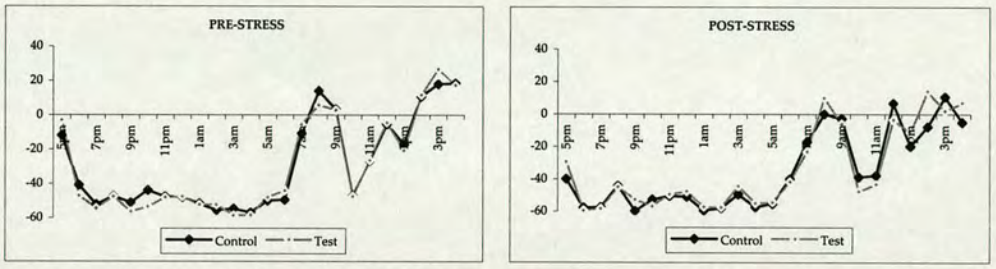


Figure 6-15: Circadian pattern of behavioural activity, pre- and post-stress[1]

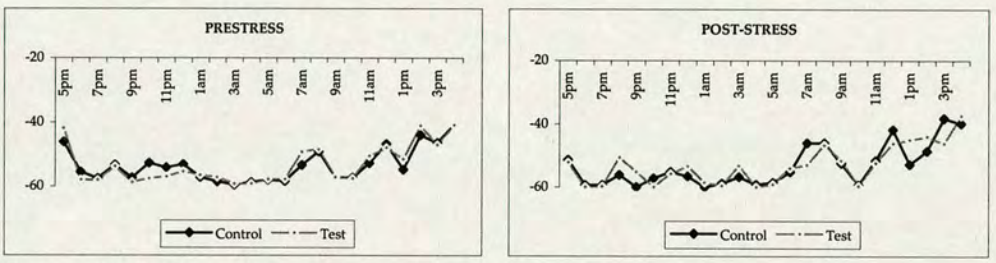


Figure 6-16: Circadian pattern of feeding, pre- and post-stress[1]

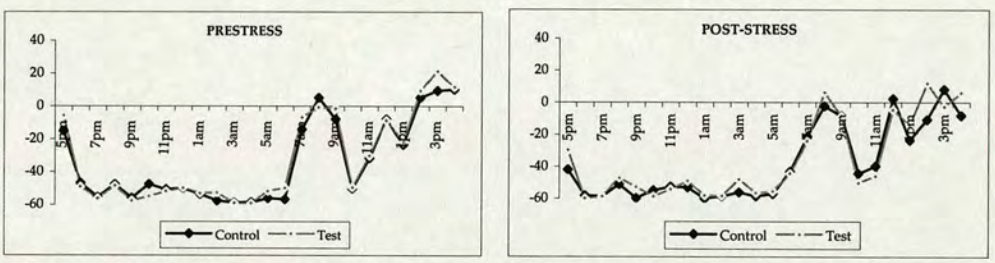


Figure 6-17: Circadian pattern of interacting with the environment, pre- and post-stress[1]

[1] In Figures 6-14 to 6-17 the x-axis is divided into hour-long bins. The y-axis extends from sixty to minus sixty. Minus sixty equals that the behaviour in question was not seen at all during the hour. Sixty indicates that the behaviour was continuous throughout the hour. Zero indicates that the behaviour was recorded for thirty scans out of sixty.

Table 6-8: Relationship (correlation percentage (p-value)) between behavioural categories and the mean salivary cortisol concentration over 24hours

Category	Correlation with mean cortisol: pre-stress	Correlation with mean cortisol: post-stress
Postures		
Lateral Lie	-16.6 (0.48)	33.7 (0.15)
Ventral Lie	20.3 (0.39)	-44.9 (0.047)
Stand	-11.4 (0.63)	33.4 (0.15)
Walk	-14.1 (0.55)	57.7 (0.008)
Behaviours		
Alert	-22.9 (0.33)	-42.1 (0.065)
Idle/Doze	58 (0.007)	4.7 (0.85)
Chew	-33.9 (0.14)	-33.7 (0.15)
Nose	-55.9 (0.01)	-18.4 (0.44)
Root	10.1 (0.67)	56.4 (0.01)
Social	-43.2 (0.057)	-32.4 (0.16)
Substrates		
None	44.2 (0.051)	-19.5 (0.41)
Straw	-22.8 (0.33)	35.6 (0.12)
Wall	-33.2 (0.15)	-23.2 (0.32)
Pig	-18 (0.45)	-23.1 (0.33)
Feeder	-37.6 (0.10)	-10.3 (0.67)
Drinker	-19.8 (0.40)	17.4 (0.46)

The only significant postural relationship with the mean cortisol value was a positive one with the amount of walking in the post-stress observations. For the behavioural categories, in the pre-stress observation animals with higher cortisol values tended to idle and doze more. There appeared to be no relationships between any of the substrate categories and the cortisol concentration.

6-3-6. Home pen behaviour - Detrended Fluctuation Analysis (DFA)

The pattern of postural activity (i.e. the sequence of standing/walking versus lying) was significantly more structured in test pigs compared to controls following the stressor period but not before (Table 6-9). However, there was a tendency for the pattern to be more structured before the stressor period. For the behavioural activity pattern there was a tendency for the fluctuation to be more structured in test animals than in controls after the stressor period but not before. None of the other DFA categories showed even a tendency to differ between treatment groups, either

before or after the stress or treatment. Further analysis of these data is presented in Section 7-2-5.

In the second observation (post-stress) there was a significant correlation (for all pigs analysed together) between the mean cortisol value over 24hours and the DFA α value for postural activity (Table 6-10 and Fig. 6-17). The correlation between these two parameters was not seen prior to the stress treatment but using a Bonferroni correction to take into account multiple tests does not make the relationship non-significant. The correlation indicates that larger mean cortisol levels were associated with increasing structure in the pattern of postural activity.

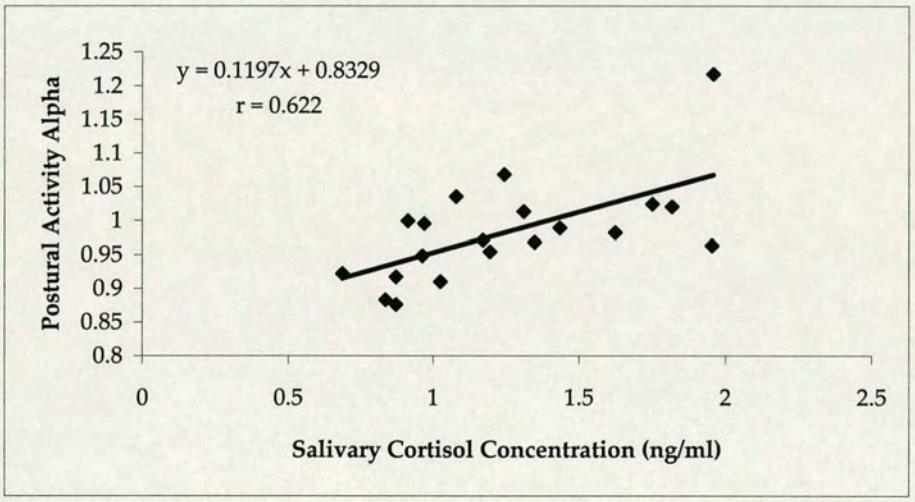


Figure 6-17: Relationship between postural activity α and salivary cortisol concentration in the post-stress observation/sample

Table 6-9: Home pen observations: Detrended Fluctuation Analysis (α: Mean ± S.D.)

DFA Category	Before stress treatment			After stress treatment			Before vs. After	
	Control	Test	Test vs. Control	Control	Test	Test vs. Control	Control	Test
Postural Activity	0.932 (0.074)	0.973 (0.041)	t=-2.02 P=0.075	0.962 (0.056)	1.004 (0.088)	t=-2.46 P=0.036	t=-1.34 P=0.212	t=-1.12 P=0.29
Behavioural Activity	1.038 (0.063)	1.069 (0.083)	t=-1.28 P=0.23	1.008 (0.051)	1.047 (0.060)	t=-1.91 P=0.089	t=1.84 P=0.099	t=0.68 P=0.51
Feed	0.761 (0.090)	0.790 (0.073)	t=-0.69 P=0.51	0.835 (0.057)	0.838 (0.093)	t=-0.09 P=0.93	t=-4.29 P=0.002	t=-2.21 P=0.054
Interact	1.040 (0.057)	1.068 (0.076)	t=-1.12 P=0.29	1.016 (0.052)	1.051 (0.055)	t=-1.68 P=0.13	t=1.64 P=0.14	t=0.57 P=0.58
Lateral	1.075 (0.064)	1.091 (0.065)	t=-0.81 P=0.44	1.068 (0.045)	1.084 (0.040)	t=-0.85 P=0.42	t=0.34 P=0.74	t=1.05 P=0.33
Pig	0.703 (0.086)	0.672 (0.071)	t=1.05 P=0.32	0.686 (0.687)	0.723 (0.055)	t=-1.55 P=0.16	t=0.77 P=0.46	t=-1.75 P=0.12
Social	0.643 (0.093)	0.646 (0.091)	t=-0.16 P=0.88	0.676 (0.075)	0.691 (0.061)	t=0.2 P=0.85	t=-1.52 P=0.16	t=-1.4 P=0.2
Straw	0.940 (0.055)	0.951 (0.088)	t=-0.43 P=0.68	0.886 (0.061)	0.903 (0.051)	t=-0.88 P=0.4	t=2.4 P=0.04	t=2.19 P=0.056

Table 6-10: Relationship (correlation percentage (p value)) between each DFA *a* and the mean salivary cortisol concentration over 24hours

DFA category	Correlation with mean cortisol: pre-stress		Correlation with mean cortisol: post-stress	
Postural Activity	36	(0.12)	62	(0.003/0.024)
Behavioural Activity	-21	(0.38)	41	(0.07/0.56)
Feed	39	(0.09/0.72)	-2	(0.94)
Interact	-21	(0.39)	42	(0.069/0.55)
Social	-30	(0.19)	0.2	(0.99)
Straw	-11	(0.66)	41	(0.07/0.56)
Lateral	23	(0.34)	-7	(0.78)
Pig	-19	(0.42)	-10	(0.68)

6-3-7. Open field behaviour

The only difference between the two groups, in the main behavioural categories (Table 6-11), was that, in the first test, control pigs directed more attention towards the gate than test pigs did. There were no difference between test and controls in the general amount of activity, or in the amount of rooting, time spent alert, general exploration, or attention directed to the novel object. There was a significant effect of repeated testing for rooting behaviour and for behaviour directed at the gate. The duration of rooting decreased over the three repetitions of the test. The time spent directing attention to the gate increased over the three tests. There was a tendency for test pigs to defecate less than controls in the first test (Table 6-12), but the total number of defecations was small. There was also a tendency for test pigs to spend more time rooting straw while walking in the first test (Table 6-13).

Table 6-11: Open field observations: main categories (Mean time in seconds ± S.D.)

	Test One			Test Two			Test Three			Repeated measures ANOVA		
	Control	Test	Test vs. Control	Control	Test	Test vs. Control	Control	Test	Test vs. Control	Treat. Effect	Time Effect	Interaction
Stand	495 (23.2)	486.6 (23.1)	t=0.82 P=0.44	489.6 (33.4)	491.1 (34.2)	t=-0.11 P=0.92	484.6 (28)	473.8 (34.8)	t=0.92 P=0.38	F=0.31 P=NS	F=1.77 P=NS	F=0.43 P=NS
Walk & Run	105 (23.2)	111.8 (21.2)	t=-0.66 P=0.53	110.4 (33.4)	108.9 (34.2)	t=0.11 P=0.92	115.4 (28)	126.2 (34.8)	t=-0.92 P=0.38	F=0.27 P=NS	F=1.84 P=NS	F=0.38 P=NS
Root	217.8 (78.2)	222 (100.4)	t=-0.1 P=0.92	161.7 (70.2)	154 (75.1)	t=0.32 P=0.76	140 (100.7)	109.4 (38.5)	t=0.77 P=0.46	F=0.18 P=NS	F=11.2 P<0.01	F=0.38 P=NS
Alert	195.7 (76.4)	221.1 (78.7)	t=-0.77 P=0.46	213.9 (80.5)	177 (100.7)	t=1.13 P=0.29	156.8 (57.3)	163.4 (75.5)	t=-0.28 P=0.79	F=0 P=NS	F=3.25 P=NS	F=1.33 P=NS
Explore	353.1 (72.6)	329.5 (72.6)	t=0.79 P=0.45	288.9 (65.4)	329.2 (114.2)	t=-1.21 P=0.26	336 (68)	316.5 (88)	t=0.82 P=0.43	F=0 P=NS	F=1.67 P=NS	F=2.05 P=NS
Gate	33.84 (15.3)	20.2 (15.5)	t=2.38 P=0.04	55.5 (51.8)	67.3 (46.9)	t=-0.54 P=0.6	96.8 (64.6)	122.9 (103.6)	t=-0.62 P=0.55	F=0.19 P=NS	F=14.3 P<0.01	F=0.84 P=NS
Object	22.5 (23.3)	10.4 (8.9)	t=1.53 P=0.16	17.5 (17.9)	24.2 (20.4)	t=-0.77 P=0.46	31 (27.1)	35.4 (29.4)	t=-0.39 P=0.71	F=0 P=NS	F=2.98 P=NS	F=1.03 P=NS
Novel object latency	121.7 (87.6)	151.2 (119.9)	t=-0.69 P=0.51	8 (12.84)	57.1 (99.8)	[1]t=-0.98 P=0.35	94.4 (121.8)	25 (51.6)	t=1.48 P=0.17	F=0.02 P=NS	F=7.46 P=NS	F=2.59 P=NS

[1]Log-transformation used, back-transformed means displayed

Table 6-12: Open field behaviour: behaviours performed while standing (Mean time in seconds ± S.D.)

	Test One			Test Two			Test Three		
	Control	Test	Test vs. Control	Control	Test	Test vs. Control	Control	Test	Test vs. Control
Defecations (total number)	3.6 (1.8)	2.4 (1)	t=1.91 P=0.09	3 (1.9)	2.9 (1.4)	t=0.13 P=0.9	3.4 (1.4)	2.4 (1.4)	t=1.63 P=0.14
Latency to defecate	118.5 (107)	208.5 (155.4)	t=-1.39 P=0.2	149.2 (182.5)	121.5 (64.3)	t=0.52 P=0.62	75.8 (71)	45 (33.7)	t=1.1 P=0.3
Stand root straw	207.2 (75.7)	201.9 (90.5)	t=0.15 P=0.89	151.2 (65.1)	144.4 (71.3)	t=0.28 P=0.78	131.9 (98.1)	103.3 (38.3)	t=0.74 P=0.48
Stand nose wall	80.3 (71.6)	75.9 (57.5)	t=0.16 P=0.87	56.7 (27.1)	85.8 (116.9)	t=-0.71 P=0.5	75.8 (53.4)	50.6 (31.3)	t=1.29 P=0.22
Stand idle	4.72 (6.1)	6.3 (5.5)	t=-0.95 P=0.37	20.8 (18.2)	21.5 (23.1)	t=-0.06 P=0.95	16.6 (8)	32.3 (56.8)	t=-0.82 P=0.43
Stand alert	147.7 (65.7)	172.7 (70.4)	t=-0.87 P=0.41	188.9 (71.5)	148.5 (83.3)	t=1.38 P=0.2	134 (44.6)	130.7 (58.7)	t=0.2 P=0.85
Stand nose door	28.1 (11.6)	15.1 (11.7)	t=2.95 P=0.016	38.8 (33.3)	39.7 (33.6)	t=-0.06 P=0.96	77.4 (46)	78.5 (49)	t=-0.04 P=0.97
Stand push door	4.5 (4.7)	4.3 (5.4)	t=0.09 P=0.93	15.9 (21.3)	27 (24.5)	t=-1.5 P=0.17	18 (24.5)	43 (59.7)	t=-1.26 P=0.24

Table 6-13: Open field behaviour: behaviours performed while walking (Mean time in seconds ± S.D.)

	Test One			Test Two			Test Three		
	Control	Test	Test vs. Control	Control	Test	Test vs. Control	Control	Test	Test vs. Control
Walk root straw	10.6 (4.4)	20.1 (11.8)	t=-2.17 P=0.06	10.5 (6.9)	9.7 (13)	t=0.23 P=0.82	8.1 (6.1)	6.1 (6.3)	t=0.86 P=0.41
Walk idle	44.8 (24.8)	43 (12.7)	t=0.21 P=0.84	73.2 (35)	68.6 (23.9)	t=0.33 P=0.75	82.5 (30.6)	85 (31.6)	t=-0.16 P=0.88
Walk alert	46.7 (15.3)	44.7 (20.1)	t=0.22 P=0.83	24.4 (14.5)	27.3 (27.1)	t=-0.41 P=0.69	22.2 (18.7)	30.6 (28.9)	t=-0.8 P=0.44

6-4. Discussion

6-4-1. Recapitulation of aims

This experiment aimed to investigate the effects of a stressor treatment involving repeated social and environmental disturbance and to measure pig behaviour under these stressors using the fractal analysis technique of Detrended Fluctuation Analysis (DFA).

6-4-2. Detrended Fluctuation Analysis (DFA) of pig behaviour

This study has clearly shown that DFA can be applied to observations of pig behaviour, providing a novel measure of behavioural complexity. The complexity of test pigs' postural activity was significantly different from that of controls following the stress treatment, with test pigs having a more structured pattern of activity. However, there was a trend towards this difference prior to the stress treatment. The test and control pigs were not allocated to treatments at random, so pre-treatment differences were not entirely unlikely. To maximise the likelihood of clear social defeats occurring, test pigs were chosen to be smaller, and therefore putatively subordinate (see section 6-4-4-2), to controls and companions. The analysis may well have identified differences, in the complexity of subordinate and dominant behaviour, that could either reflect different levels of stress or, alternatively, intrinsic differences that have no influence on the animals' welfare (e.g. physical size or adjustments in behaviour due to social status). Irrespective of the causation of the difference between test and control animals, it is significant that no such difference exists in the total amount of activity or in the circadian pattern of activity. So, as previously shown for Ibex vigilance and feeding behaviour (Alados et al. 1996) and for chicken vigilance behaviour (Chapter Four), a fractal analysis in the form of DFA revealed differences in behaviour between different groups of animal where standard analysis methods did not.

It is interesting that the mean cortisol concentration over 24h and the α for postural activity (PAα) are highly significantly correlated in the post-stress values. Both cortisol and PAα also differed significantly between test and control groups after the stressor treatment but not before and in both cases there was a suggestion of a pre-treatment difference. This result does at least suggest that there may be a

link between the cortisol output of an animal and its fractal behavioural complexity. This result deserves further study as establishing physiological correlates of fractal complexity in behaviour would be a major (and necessary) step towards the validation of fractal analysis as a stress assessment tool. The decreasing behavioural complexity with increasing cortisol concentration would match Alados' (Alados et al. 1996; Alados & Weber 1999; Alados & Huffman 2000) view that stress causes a decrease in behavioural complexity. To avoid any behavioural disturbance[2] caused by saliva sampling, yet to still be able to collect simultaneous behavioural and physiological data would require either catheterised animals (Carroll et al. 1999; Fudge et al. 2002) or the use of a saliva collection device (Schonreiter et al. 1999).

6-4-3. Effects of the stressor treatment

Although the application of a social defeat was variable (some animals were repeatedly and clearly defeated, while two pigs were not defeated during any of the interactions), most interactions involved aggression and some of the undecided contests involved the most aggression. These can still be regarded as imposing social stress (as backed up by the increased cortisol concentration seen in intruder pigs) and the other parts of the stressor treatment were successfully applied. Despite this, the stressor treatment as a whole seems to have had no major long-term effect on the animals, at least according to the range of measures that were made here.

There was an apparent decrease in the weight gain of test pigs over the course of the stress period compared to controls. Since there was a marked difference in body weight between test and control pigs, this only became apparent when starting weight was used as a covariate in the analysis. The residents showed a lower growth rate than test or control pigs over the stressor period. This could be due to stress in the resident animals. One resident from each pen was in an aggressive interaction every two days and sometimes these (subjectively) appeared as stressful for the resident as for the test pig, although the residents did not show an increase in cortisol following the interaction, while the intruders did. Even removing pigs from a pen for weighing can significantly affect their feed intake on

[2] It was felt that the behavioural disturbance of saliva sampling was minimal. During the day inactive pigs would sometimes get up and come to the front of the pen at the sampling point but they soon returned to their inactivity. This would have little effect on the overall total of behaviour but could affect the fractal measures.

the same day (Augspurger & Ellis 2002). It is not known if pigs compensate with later increases in intake following such a disturbance, but it is at least possible that the regular (every two days) weighing of the residents could have affected their growth rate.

Although mean cortisol level over a 24 hour period was significantly higher in test pigs following the treatment but not before, there is a suggestion that the test pigs tended to have higher values in the pre-treatment samples. Test pigs were putatively subordinate to controls. In other studies, subordinate pigs have been found to have higher cortisol concentrations than dominants. De Jonge and associates (1996) reared pigs either in an impoverished or an enriched environment. They found that in the impoverished environment (close to the rearing condition used here) subordinate pigs had higher basal levels of cortisol than dominants, whereas when animals where reared in an enriched environment subordinates and dominants had similar levels. The relationship between social status and cortisol levels is variable, however. Ruis and colleagues (2002) found that subordinate animals had a higher cortisol response to mixing than dominants. However, Otten and co-workers (1999) found that high-ranking animals introduced into unfamiliar groups had a higher cortisol response than lower ranking pigs. The cortisol response to the aggressive interactions was only measured once in this study but it was found that the test pigs showed an acute cortisol response, while the resident animals did not. In primates, the cortisol concentration of subordinates relative to dominants depends on social organization (Abbott et al. 2003) and particularly the frequency of social stress and opportunities for social support.

The difference between test and control pigs in the post-stress sample appears to be due to increased values in the afternoon, suggesting a possible alteration in rhythm. However, with reference to the literature it could be argued that such a pattern of release represents a more normal state than that seen in the controls. A similar U-shaped cortisol profile was found by de Jong and colleagues (2000b) in a group of pigs housed in an enriched environment compared to a group housed in a barren environment. De Groot and co-workers (2000) showed that there was some degree of variability: in two of three replicates there was a U-shaped response[3], with the afternoon peak much larger than the morning peak in one, while in a third

[3] These two replicates were combined in de Jong et al.'s (2000b) analysis and figures.

replicate (not analysed by de Jong et al. 2000b) there was less of a clear U-shaped profile. Ruis and colleagues (1997) show examples of cosinor fits to their cortisol rhythm data (Fig. 1 in their paper). However, visual inspection of these examples suggests a U-shaped profile would provide a better description of the data. In human medicine there is a growing recognition that a flattened cortisol profile can be an indicator of stress or a predictor of future mortality in cancer patients (Sephton et al. 2000; Miller et al. 2002).

There were few behavioural differences between test and control pigs in either the home pen or the repeated open field tests. In the home pen the total activity of the two groups did not differ. However, the form of inactive posture altered, with test pigs significantly increasing the time they spend lying on their side and therefore spending significantly less time lying on their front than the control animals. Lateral lying could be considered as a more passive posture than ventral lying. However, the lack of any alteration in general activity, exploration, social behaviour or feeding makes it difficult to conclude anything else than that the test pigs were not behaviourally depressed. It is possible that larger alterations in behaviour might have been seen in the test pigs during, or immediately after, the stressor treatment. The second home pen observation started five days following the test pigs' return to the home pen after the final defeat. This gap was deliberately inserted because the focus was on assessing chronic effects of the treatment, rather than any lingering acute effects. Paradoxically, it is also the case that alterations in behaviour could have become apparent after the second observation: in rats a single social defeat increased immobility in a sudden silence test, three and four weeks following a single social defeat but not one or two weeks after the defeat (Koolhaas et al. 1990).

6-4-4. Discussion of the method

6-4-4-1. Attack latency and subsequent aggressive interactions

One aim of this experiment was to assess the effects of a stressor treatment, involving repeated social defeat plus sensory contact and additional stressors, on the behaviour and physiology of growing pigs. This treatment was loosely based around animal models of depression and the aim had been to attempt to develop a

model of depression in pigs. However, although the residents were pre-selected to have low attack latency (an indication of aggressive tendency: Erhard et al. 1997; D'Eath 2002), many of the interactions failed to result in a clear defeat.

One flaw in the experiment is that only residents were tested in the attack latency test, with short attack latency (SAL) pigs being chosen for later use. Erhard and co-workers (1997) mixed groups of four pigs in three combinations: SAL with SAL, SAL with long attack latency (LAL) and LAL with LAL. The degree of aggression shown during these mixings depended on the particular combination. In the SAL-LAL combination less overt aggression was seen because the LAL pigs withdrew from any interaction. The longest and most intense fighting was seen in the SAL-SAL combination. In the current experiment, the resident animal was SAL but whether the intruder had a high or low aggressive tendency was essentially left to chance. Although the residents initiated the majority of aggression the resulting behaviour of the intruder was likely dependent on whether it had an inherently high or low aggressive tendency. In rodent studies, although aggressive individuals fight less as an intruder than as a resident, they still fight more than non-aggressive individuals (van Oortmerssen et al. 1985). Using groups of mice bred selectively on the basis of attack latency to create groups of reliably SAL or LAL animals (Benus et al. 1991), it has recently been shown that LAL mice are more susceptible to chronic social stress in a defeat and sensory contact model than SAL mice (Veenema et al. 2003).

Although Koolhaas and colleagues (1990) suggested that animals with an active coping pattern prior to a defeat were affected more than animals with a passive coping style, (with this difference being more important in determining long-term effects than the severity of the defeat) this finding has not been supported by subsequent work, which found that increased severity of fighting by the intruder (i.e. when it was more active in the interaction) lessened the apparent effects of the defeat (Meerlo et al. 1999; Veenema et al. 2003). Meerlo and associates (1999) propose that those animals that fought back, putatively did not view the encounter as being a defeat, while those animal that submitted more easily did.

The two main determinants of the outcome of a contest between unfamiliar pigs are weight and inherent aggressive tendency (D'Eath 2002). D'Eath found that weight was the best predictor of the outcome of aggressive interactions, while the

inherent aggressiveness of the combatants affected the form and duration of the interaction. Highly aggressive pigs persist in their aggression and there is more bullying in groups of aggressive individuals. It may be that although the residents had a weight advantage, many of the intruders were aggressive themselves, resulting in an interaction that failed to reach a clear conclusion (i.e. a defeat). In the attack latency test a 'priming' effect is commonly found (Erhard & Mendl 1997; D'Eath & Burn 2002), i.e. attack latency is decreased in the second of two tests. This effect was also seen in both pairs of tests carried out here. However, the intruder animals may have also undergone a priming effect such that the repeated tests were effectively training then to be aggressive. The fact that the total number of successful defeats decreased over the five tests supports this possibility. It could also be that the weight advantage of the residents actually became a handicap in the relatively small arena used here.

6-4-4-2. Weight, social rank and the potential effects of social defeat

Rather than randomly assigning pigs to be test or controls, smaller males were deliberately picked as test animals. The reasons for this were mainly practical. With the range of litters available for use, if the larger experimental pigs had been used there would have been a much smaller weight difference between test and resident pigs, reducing the chance of clear defeats occurring. Size was used as a putative correlate of rank. Although weight may not predict rank when pigs are initially mixed (Meese & Ewbank 1973), it is possible that weight differences within a litter are partly due to dominance relationships (McBride et al. 1964) and so larger individuals are likely to be dominant to smaller ones within a litter. Forkman and co-workers (1995) found that, within litter groups, a ranking of pigs on the basis of weight showed a high correlation($r=0.76$) with a ranking based on a competitive feeding test.

A better method to assess the rank relationship would have been to use a competitive feeding test (e.g. Beilharz & Cox 1967b). However, even given a more accurate assessment of rank, the decision of whether to then choose the dominant or subordinate as the test animal is not straightforward. Ideally, a larger experiment could allocate test animals evenly between dominant and subordinate. This would allow the influence of original social status on the effects of defeat (and/or social

support) to be investigated. It is possible that the effects of repeated defeat would be greater in a previously dominant animal (i.e. loss of status model of depression; Willner et al. 1995, see also Otten et al. 2002 and section 6-4-4-4 below). However, it might be equally possible that a dominant individual receives more social support upon returning to the home pen where it is still dominant. The fact that pigs were not isolated and so were putatively socially supported (e.g. Ruis et al. 1999, 2001) may explain the lack of a large treatment effect in this experiment. Ewbank and Meese (1971) found that low ranking animals were more likely than dominant animals to be attacked after a period away from the home pen. It may be more likely that the effects of a defeat are lessened for a previously dominant animal if it returns to its home pen and is accepted back as the dominant. Since the effect of original social status is unknown the decision to try and use subordinates only was justified, although this did create problems in assessing treatment effects relative to controls.

What is actually viewed as a social defeat by the animals is uncertain. For example, in Erhard and co-worker's (1997) mixing experiment; are the LAL pigs that show avoidance when attacked by an SAL pig really being defeated? Or are the only pigs that might truly be considered to be defeated whichever of the SAL pigs eventually lost out in the highly aggressive contests with other SAL pigs? To put it more simply, is it possible for an animal to experience defeat if it does not try to win in the first place? Pigs recorded as having no-success in social interactions (e.g. that were always displaced by other animals) were actually less stressed than pigs with intermediate success (i.e. those that were actively challenging other animals but with only intermittent success) (Mendl et al. 1992). The authors suggest that the low aggression of the no success group represented a distinct strategy for coping with the social environment. In most studies involving social defeat the definition used involves the intruder showing submissive behaviours (e.g. Koolhaas et al. 1990), with no requirement of resistance.

6-4-4-3. Defence or submission in defeated pigs

There has been some debate over whether pigs show truly submissive behaviours or whether the behaviours shown by losing animal are more properly considered as defensive. A submissive behaviour might be defined as one that is intended as a signal to the other animal that the actor has given up in order to stop the aggression

of the victor. Defensive behaviours on the other hand are not clearly intended as signals, being more practical attempts by the animal to limit the effects of an opponent's aggression. McBride and associates (1964) described submissive behaviours in pigs. They suggested that avoidance alone would not be enough to inhibit aggression in a pen of pigs and that submissive pigs signalled their submission by lowering their eyes and by squealing. However, other authors have struggled to identify clear-cut true submissive behaviours in pigs (Rushen & Pajor 1987). Ewbank and Meese (1971) suggested, "the pig does not possess a ready means of inhibiting aggression but simply depends on the ability to flee from the aggressor". It may well be that pigs do signal subordination in ways too subtle for observers to clearly record them. Fraser and Broom (1997) suggest that some of the earlier observations of social hierarchies in pigs may be flawed because observers missed subtle social behaviours, such as 'head-tilting' (an avoidance-submission behaviour) or 'aiming' which occurs as " an upward lift of the head in the direction of a threatened conspecific at a distance of 2-3m". Although truly submissive behaviours are not obvious in pigs, subordinate behaviour occurs in the form of fleeing, turning away from attacks or standing in a particular drooped posture with an arched back (Meese & Ewbank 1973). Fraser and Broom (1997) suggest that social systems in housed pigs may be better described as an avoidance order rather than a dominance order maintained through aggression. They suggest that "avoidance behaviour seems to diminish aggressive outcome in social interactions" and "if the available area is too small to permit the animals to perform subordination behaviour this will cause settled pair-relations to break down in frustration".

Fighting at mixing occurs for two reasons (Rushen 1988; D'Eath 2002). Firstly, pigs are motivated to drive off unfamiliar animals. Secondly, aggression occurs as a new social hierarchy is formed. In these different situations, differences in the persistence of aggression in the face of submissive behaviour might be predicted, i.e. hierarchical aggression should stop when submission is seen whereas territorial aggression (where the aim is to cause the other animal to leave) should not necessarily stop when submission is seen. It seems reasonable to say that if aggression and defeat might induce depression it would be in a social context (i.e. hierarchical) rather than a territorial context. In a territorial context the behavioural symptoms of depression would not be adaptive. The resident-intruder test is set up

to mimic an incursion into the resident's territory and resultant territorial defence by the resident. It this case it might be predicted that aggression would possibly continue beyond the display of submissive behaviour. However, from the point of view of the intruder a defeat by an unfamiliar animal in a territorial battle might well have different psychological consequences to defeat by a familiar group member in a hierarchical context. The social theories of depression (Price 1967; Price et al. 1994; Sloman & Gilbert 2000) view depression as being a consequence of a negative social experience when the individual has to re-assess their role and withdraw from further competitive interactions.

There are also implications for the method. If pigs lack submissive behavioural signals they have no way of inhibiting the aggression of the winner (Rushen & Pajor 1987). Forcing the losing animal to remain in such close contact with the winner could have been such an artificial situation that it may have effectively forced losers back into offence rather than defence. McGlone and Curtis (1985) found that pigs in pens with hide areas where they could stick their head to avoid attacks had a lower level of agonistic behaviour following mixing. This was due to losing pigs in aggressive encounters breaking off the interaction and entering the hide area.

6-4-4-4. Alternative methods for future experiments

Although the treatment can be assessed in general stress terms the stressors used did not provide a model of depression. What are the options for future experiments relating to investigations of depression in pigs? One option would be to refine the current format. A larger arena and hide areas (e.g. McGlone & Curtis 1985) might help ensure a more reliable defeat. Within this format it might be worthwhile to move to a repeated defeat/sensory contact model closer to the tree shrew model (Fuchs & Flügge 2002), in which the test animal is repeatedly exposed to the same dominant animal. This situation may be more likely to create an on-going situation of negative self-appraisal, in terms of the animal's likely chances of winning a social interaction. Rushen and Pajor (1987) brought two initially unacquainted young pigs together for one hour on successive days. On the second day, aggressive interactions were greatly decreased due to a decrease in offensive behaviour and an increase in defensive by the day one loser (see also Rushen 1988). This shift in behaviour is most probably the result of a shift in the loser animal's assessment of its chances of

success. Such a model would also remove any potentially confounding effects of social support.

A simple way of guaranteeing a defeat would be to mix an intruder with two residents or simply to use a more realistic mixing model. Recently, Otten and colleagues (2002) described the results of an experimental mixing. In this experiment, groups of 12-week-old pigs were created and the dominant within each group identified. These dominant animals were then removed from the group and housed singly for two to three weeks before being returned to their old groups. In the interactions that followed this return some animals re-gained their dominant status whilst some did not. In the first few hours following the re-grouping the animals that failed to re-gain dominant status spend less time (relative to those that re-gained their previous status) exploring the pen and were less active generally, spending more time lying down. In this study the losing animals did show what might be described as a depressed behavioural profile on return to their old groups. However, the behavioural differences only persisted over the first few hours after the re-grouping, so any state of depression appears transient. The behaviour of previously dominant animals differed from previous low-ranking animals in the same situation (Otten et al. 1999), so it is not necessarily the defeat(s) *per se* that caused the behavioural inhibition but the loss of status. The animals were only studied over the ten hours after the re-grouping so it is not known if the loser showed any long-term changes in behaviour, physiology or growth rate that might indicate longer-term effects of losing their status.

6-5. Conclusion

In conclusion, although the exact form of stressor treatment intended to occur did not actually occur, the experiment as outlined did apparently prove mildly stressful to the test pigs. Although there were minimal behavioural effects of the treatment the use of Detrended Fluctuation Analysis did identify differences between test and control pigs that were not apparent in more standard summary measures. The use of DFA as a measure of temporal organisation in pig behaviour may prove useful in future studies.

Chapter Six Appendix

Table A6-1: Attack-latency test number one[1]

Resident				Intruder						
ID	Sex	Litter	Weight (Kg)	ID	Sex	Litter	Weight (Kg)	Intruder weight % of Resident	Nose latency (Sec.)	Attack latency[2] (Sec.)
849	(F)	1	38.5	881	(M)	4	25.5	66.2	30	NA
851	(M)	1	37	865	(F)	5	24.5	66.2	38	NA
852	(F)	1	41	911	(F)	8	26	63.4	4	103
853	(M)	1	38	868	(M)	5	25	65.8	3	51
854	(F)	1	43.5	890	(F)	6	27	62.1	10	NA
855	(F)	2	33.5	866	(F)	5	22	65.7	54	137
856	(M)	2	44	846	(F)	1	29.5	67.1	10	NA
857	(M)	3	39.5	845	(F)	1	25	63.3	46	277
858	(M)	3	37	872	(F)	5	25	67.6	105	NA
859	(F)	3	40	848	(F)	1	28.5	71.3	10	49
860	(M)	3	42	850	(F)	1	32.5	77.4	40	217
862	(M)	3	37	867	(F)	5	24	64.9	66	NA
863	(M)	3	36	882	(M)	4	32	88.9	24	203
864	(M)	3	39	868	(M)	5	25	64.1	90	NA
876	(M)	4	34	896	(M)	7	22.5	66.2	7	71
878	(M)	4	40	911	(F)	8	26	65	216	36
879	(M)	4	33.5	783	(M)	11	19	56.7	25	121
880	(M)	4	34.5	887	(F)	6	24	69.6	71	I64
882	(M)	4	32	793	(F)	9	19.5	60.9	18	Stop
884	(F)	4	32	906	(M)	7	18.5	57.8	10	125
869	(F)	5	27	787	(F)	11	15.5	57.4	21	NA
871	(M)	5	31.5	881	(M)	4	25.5	81.0	9	291
873	(F)	5	27.5	793	(F)	9	19.5	70.9	68	I207
874	(M)	5	27.5	783	(M)	11	19	69.1	12	NA
875	(F)	5	28.5	903	(F)	7	21.5	75.4	18	NA
	Mean	35.8					24.1	67.4	40.2	140.1[3]
	S.D.	5					4.2	7.3	46.3	88.0
	Range	27-44					19-32.5	56.7-88.9	3-216	36-291

	Resident Attacks	12/25
	Intruder Attacks	2/25
Chosen Residents	Attacks	9/14
	Mean latency	156.2
	S.D.	95.7

[1] The results from those pigs that were subsequently picked as aggressive resident are shaded in grey.

[2] Instances were the intruders attacked first are shown as I220 in the attack latency column. NA = No Attack. Stop = interaction was stopped before an attack occurred or the time ran out (e.g. due to excessive mounting or escape attempts).

[3] Mean latency is calculated for attacking pigs only.

Table A6-2: Attack-latency test number two[1]

Resident				Intruder						
ID	Sex	Litter	Weight (Kg)	ID	Sex	Litter	Weight (Kg)	Intruder weight % of Resident	Nose latency (Sec.)	Attack latency[2] (Sec.)
849	(F)	1	38.5	870	(F)	5	25.5	66.2	4	280
851	(M)	1	37	887	(F)	6	24	64.9	9	48
852	(F)	1	41	877	(M)	4	26.5	64.6	1	NA
853	(M)	1	38	798	(F)	9	24	63.2	3	157
854	(F)	1	43.5	914	(M)	8	31	71.3	4	I59
855	(F)	2	33.5	798	(F)	9	24	71.6	6	28
856	(M)	2	44	847	(F)	1	30.5	69.3	3	I82
857	(M)	3	39.5	867	(F)	5	24	60.8	11	133
858	(M)	3	37	866	(F)	5	22	59.5	5	NA
859	(F)	3	40	872	(F)	5	25	62.5	7	34
860	(M)	3	42	870	(F)	5	25.5	60.7	5	NA
862	(M)	3	37	845	(F)	1	25	67.6	38	NA
863	(M)	3	36	865	(F)	5	24.5	68.1	18	30
864	(M)	3	39	890	(F)	6	27	69.2	5	NA
876	(M)	4	34	867	(F)	5	24	70.6	6	NA
878	(M)	4	40	870	(F)	5	25.5	63.8	15	29
879	(M)	4	33.5	905	(F)	7	25	74.6	8	186
880	(M)	4	34.5	903	(F)	7	21.5	62.3	6	175
882	(M)	4	32	906	(M)	7	18.5	57.8	7	NA
884	(F)	4	32	783	(M)	11	19	59.4	15	24
869	(F)	5	27	883	(M)	4	21	77.8	4	NA
871	(M)	5	31.5	896	(M)	7	22.5	71.4	14	52
873	(F)	5	27.5	787	(F)	11	15.5	56.4	41	120
874	(M)	5	27.5	906	(M)	7	18.5	67.3	6	NA
875	(F)	5	28.5	793	(F)	9	19.5	68.4	35	I137
	Mean	35.8					23.6	66.0	11.0	99.7
	S.D.	5					3.6	5.4	11.0	81.9
	Range	27-44					19-31	56.4-77.8	1-41	24-280

	Resident Attacks	13/25
	Intruder Attacks	3/25
Chosen Residents	Attacks	8/14
	Mean latency	56.3
	S.D.	44.3

[1] The results from those pigs that were subsequently picked as aggressive resident are shaded in grey.

[2] Instances were the intruders attacked first are shown as I220 in the attack latency column. NA = No Attack. Stop = interaction was stopped before an attack occurred or the time ran out (e.g. due to excessive mounting or escape attempts).

[3] Mean latency is calculated for attacking pigs only.

Table A6-3: Attack-latency test number three[1]

Resident				Intruder						
ID	Sex	Litter	Weight (Kg)	ID	Sex	Litter	Weight (Kg)	Intruder weight % of Resident	Nose latency (Sec.)	Attack latency[2] (Sec.)
849	(F)	1	64	881	(M)	4	44	68.8	3	368
851	(M)	1	61	866	(F)	5	43	70.5	2	NA
852	(F)	1	65	868	(M)	5	49	75.4	4	35
853	(M)	1	65	890	(F)	6	50	76.9	4	Stop
854	(F)	1	70	908	(M)	8	56	80.0	5	35
855	(F)	2	55	798	(F)	9	44.5	80.9	0	7
856	(M)	2	72.5	861	(F)	3	54	74.5	3	229
857	(M)	3	60	877	(M)	4	47	78.3	22	99
858	(M)	3	64	895	(F)	7	44.5	69.5	18	45
859	(F)	3	65	867	(F)	5	45	69.2	5	20
860	(M)	3	66	846	(F)	1	53	80.3	14	383
862	(M)	3	60	911	(F)	8	45	75	16	513
863	(M)	3	60	872	(F)	5	49	81.7	8	10
864	(M)	3	62	793	(F)	9	42.5	68.6	11	NA
876	(M)	4	59	865	(F)	5	44	74.6	5	569
878	(M)	4	65	847	(F)	1	53	81.5	11	21
879	(M)	4	56	906	(M)	7	37.5	67.0	5	STOP
880	(M)	4	58	903	(F)	7	42.5	73.3	8	NA
884	(F)	4	57	887	(F)	6	47.5	83.3	3	43
869	(F)	5	53	904	(M)	7	42.5	80.2	4	NA
871	(M)	5	56	883	(M)	4	39	69.6	32	103
873	(F)	5	54	783	(M)	11	39.5	73.2	39	23
874	(M)	5	50	787	(F)	11	31	62	9	NA
875	(F)	5	52	898	(M)	7	31.5	60.6	27	34
	Mean	60.4					44.8	74	11	149.2
	S.D.	5.7					6.3	6.3	10.3	189.1
	Range	50-72.5					31-56	60.6-83.3	0-39	7-569

	Resident Attacks	17/24
	Intruder Attacks	0
Chosen Residents	**Attacks**	14/14
	Mean latency	150.0
	S.D.	195.8

[1] The results from those pigs that were subsequently picked as aggressive resident are shaded in grey.

[2] Instances were the intruders attacked first are shown as I220 in the attack latency column. NA = No Attack. Stop = interaction was stopped before an attack occurred or the time ran out (e.g. due to excessive mounting or escape attempts).

[3] Mean latency is calculated for attacking pigs only.

Table A6-4: Attack-latency test number four[1]

Resident				Intruder				Intruder weight % of Resident	Nose latency (Sec.)	Attack latency[2] (Sec.)
ID	Sex	Litter	Weight (Kg)	ID	Sex	Litter	Weight (Kg)			
849	(F)	1	64	904	(M)	7	42.5	66.4	3	68
851	(M)	1	61	865	(F)	5	44	72.1	1	NA
852	(F)	1	65	883	(M)	4	39	60.0	5	59
853	(M)	1	65	868	(M)	5	49	75.4	3	I22
854	(F)	1	70	870	(F)	5	53	75.7	4	47
855	(F)	2	55	904	(M)	7	42.5	77.3	5	19
856	(M)	2	72.5	846	(F)	1	53	73.1	4	Stop
857	(M)	3	60	903	(F)	7	42.5	70.8	6	91
858	(M)	3	64	887	(F)	6	47.5	74.2	4	21
859	(F)	3	65	895	(F)	7	44.5	68.5	4	16
860	(M)	3	66	891	(M)	6	43.5	65.9	5	20
862	(M)	3	60	904	(M)	7	42.5	70.8	4	23
863	(M)	3	60	883	(M)	4	39	65.0	25	23
864	(M)	3	62	881	(M)	4	44	71.0	11	195
876	(M)	4	59	896	(M)	7	43.5	73.7	5	84
878	(M)	4	65	845	(F)	1	51	78.5	1	7
879	(M)	4	56	895	(F)	7	44.5	79.5	7	Stop
880	(M)	4	58	891	(M)	6	43.5	75.0	1	47
884	(F)	4	57	866	(F)	5	43	75.4	5	15
869	(F)	5	53	898	(M)	7	31.5	59.4	11	183
871	(M)	5	56	896	(M)	7	43.5	77.7	23	25
873	(F)	5	54	798	(F)	9	44.5	82.4	16	126
874	(M)	5	50	898	(M)	7	31.5	63.0	14	NA
875	(F)	5	52	787	(F)	11	31	59.6	5	10
	Mean	60.4					43.1	71.3	7.2	56.8
	S.D.	5.7					5.8	6.5	6.4	56.5
	Range	50-72.5					31-53	59.4-82.4	1-25	7-195

	Resident Attacks	19/24
	Intruder Attacks	1/24
Chosen Residents	**Attacks**	13/14
	Mean latency	36.9
	S.D.	37.6

[1] The results from those pigs that were subsequently picked as aggressive resident are shaded in grey.

[2] Instances were the intruders attacked first are shown as I220 in the attack latency column. NA = No Attack. Stop = interaction was stopped before an attack occurred or the time ran out (e.g. due to excessive mounting or escape attempts).

[3] Mean latency is calculated for attacking pigs only.

Table A6-5: Aggressive interaction Number One

Date	Pen	Test pig weight (Kg)	Resident ID (sex)	Resident weight (Kg)	Test pig weight % of Resident	Result[1]
16/1	1	61	E1 (F)	76	80.3	No defeat
16/1	2	53	C1 (F)	65	81.5	Defeat
16/1	3	54	D1 (M)	70	77.1	Defeat
16/1	4	55	A2 (F)	75	73.3	No defeat
22/1	5	44.6	C1 (F)	71.4	62.5	Defeat
18/1	6	41	C1 (F)	67	61.2	Defeat
18/1	8	52	B1 (M)	68	76.5	Defeat
18/1	9	54	E2 (M)	77	70.1	Defeat
18/1	10	57	D2 (M)	79	72.2	Defeat
16/1	11	45	B1 (M)	65	69.2	Defeat
				Mean	72.4	8/10
				S.D.	6.9	
				Range	61.2-81.5	

[1] The result of the interaction recorded here is based on a subjective assessment of the result at the time of the interaction. A defeat was recorded when the test pig showed submissive/defensive behaviours and ceased to attack the resident pig.

Table A6-6: Aggressive interaction Number Two

Date	Pen	Test pig weight (Kg)	Resident	Resident weight (Kg)	Test pig weight % of Resident	Result[1]
20/1	1	67	C2 (M)	82	81.7	No defeat
20/1	2	56.5	E2 (M)	77	73.4	No defeat
20/1	3	59	A2 (F)	82	72.0	Defeat
20/1	4	58.5	D2 (M)	83	70.5	No defeat
26/1	5	45.8	B2 (F)	74	61.9	Defeat
22/1	6	45.7	B2 (F)	72	63.5	Defeat
22/1	8	55.6	E3 (M)	83.6	66.5	Defeat
22/1	9	58.8	D3 (M)	86.2	68.2	No defeat
22/1	10	58.8	A2 (F)	85.2	69.0	Defeat
20/1	11	50	B3 (F)	67	74.6	No defeat
				Mean	70.1	5/10
				S.D.	5.8	
				Range	61.9-81.7	

[1] The result of the interaction recorded here is based on a subjective assessment of the result at the time of the interaction. A defeat was recorded when the test pig showed submissive/defensive behaviours and ceased to attack the resident pig.

Table A6-7: Aggressive interaction Number Three

Date	Pen	Test pig weight (Kg)	Resident	Resident weight (Kg)	Test pig weight % of Resident	Result[1]
24/1	1	72.8	D3 (M)	87.4	83.3	No defeat
24/1	2	60	A2 (F)	87.8	68.3	Defeat
24/1	3	63.6	B1 (M)	74.8	85.0	No defeat
24/1	4	63.4	E3 (M)	83.2	76.2	No defeat
30/1	5	46.4	A3 (F)	83.2	55.8	Defeat
26/1	6	48.9	D1 (M)	82.2	59.5	Defeat
26/1	8	56.2	C1 (F)	74.8	75.1	Defeat
26/1	9	61.8	A2 (F)	88.4	69.9	No defeat
26/1	10	62.4	E2 (M)	86.8	71.9	No defeat
24/1	11	54	C1 (F)	73.6	73.4	No defeat
				Mean	71.8	4/10
				S.D.	9.2	
				Range	55.8-85	

[1] The result of the interaction recorded here is based on a subjective assessment of the result at the time of the interaction. A defeat was recorded when the test pig showed submissive/defensive behaviours and ceased to attack the resident pig.

Table A6-8: Aggressive interaction Number Four

Date	Pen	Test pig weight (Kg)	Resident	Resident weight (Kg)	Test pig weight % of Resident	Result[1]
28/1	1	75.2	B1 (M)	81	92.8	No defeat
28/1	2	62.8	D1 (M)	86.6	72.5	No defeat
28/1	3	69.2	E3 (M)	89	77.8	Defeat
28/1	4	66	C2 (M)	98	67.4	No defeat
3/2	5	50.6	D1 (M)	89.4	56.6	Defeat
30/1	6	51.8	E3 (M)	91.2	56.8	Defeat
30/1	8	57.8	D3 (M)	94	61.5	Defeat
30/1	9	66.2	B1 (M)	82	80.7	No defeat
30/1	10	65.8	C1 (F)	77.4	85.0	Defeat
28/1	11	61	A1 (M)	86.2	70.8	No defeat
				Mean	72.2	5/10
				S.D.	12.1	
				Range	56.6-92.8	

[1] The result of the interaction recorded here is based on a subjective assessment of the result at the time of the interaction. A defeat was recorded when the test pig showed submissive/defensive behaviours and ceased to attack the resident pig.

Table A6-9: Aggressive interaction Number Five

Date	Pen	Test pig weight (Kg)	Resident	Resident weight (Kg)	Test pig weight % of Resident	Result[1]
1/2	1	78.4	B1 (M)	85	92.2	No defeat
1/2	2	66	A3 (F)	84.2	78.4	No defeat
1/2	3	71.8	C2 (M)	102	70.4	Defeat
1/2	4	70.4	E1 (F)	95.8	73.5	No defeat
	5					
3/2	6	55.4	E2 (M)	95.8	57.8	No defeat
3/2	8	61.6	A2 (F)	97	63.5	No defeat
3/2	9	69.4	C1 (F)	82.2	84.4	Defeat
3/2	10	69.6	B1 (M)	86.8	80.2	Defeat
1/2	11	60.4	D3 (M)	95.2	63.5	No defeat
				Mean	73.8	3/9
				S.D.	11.2	
				Range	57.8-92.2	

[1] **The result of the interaction recorded here is based on a subjective assessment of the result at the time of the interaction. A defeat was recorded when the test pig showed submissive/defensive behaviours and ceased to attack the resident pig.**

Chapter Seven

Further investigation of the Detrended Fluctuation Analysis methodology and the experimental data

Abstract

The Detrended Fluctuation Analysis (DFA) methodology has proven to be a useful method for describing the behavioural patterns of various species. In previous chapters the method was applied to various behavioural patterns in chickens and pigs. In this chapter the method is explored in further detail using the data generated in the three experiments. Firstly, the DFA of the experimental data was described in more detail. Secondly, the experimental data were used to investigate the properties of DFA and how they are affected by alterations in observation length and resolution.

The analysis showed that the DFA values for vigilance ($V\alpha$) and activity ($A\alpha$) differed from each other in both chicken experiments. Activity was recorded as being far more randomly structured than vigilance. This could be a genuine reflection of underlying behavioural organisation or could be an artefact of the DFA method. In both experiments, neither $V\alpha$ nor $A\alpha$ consistently correlated with total behavioural duration. So, the fractal measures do appear to provide an independent measure with which to describe behaviour. However, $V\alpha$ was consistently related to the frequency of vigilant behaviours, although this parameter did not reveal differences between treatments in the acute stress experiment. In experiment two, $V\alpha$ was found to be consistent across repeated observations for individual animals, suggesting that it may represent a consistent behavioural trait of individual animals.

In experiment three, although the α values within individual behavioural categories did not correlate with the total number of scans recorded, less frequent behaviours were found to be more randomly structured. Although, feeding behaviour was significantly more structured in the second observation compared to the first, the α values for the two observations were significantly correlated indicating that individuals maintained their relative position within the group.

When observation time was systematically reduced $V\alpha$ remained correlated with the full-length observation values, but the mean and variability increased. So, at shorter observations the calculated α will, not surprisingly, suggest a more structured behavioural pattern but sampling variability is increased.

Reducing resolution (i.e. decreasing sampling frequency) had a similar but opposite effect. As resolution decreased $V\alpha$ remained correlated with the highest resolution but the mean decreased and became more variable. Less frequent

sampling therefore results in a behavioural pattern being recorded as more randomly structured than it would be at higher resolutions.

7-1. Introduction

The aim of this chapter was to investigate Detrended Fluctuation Analysis (DFA) further using the data generated in the three experiments described in Chapters Four, Five and Six. In the first section the results of the DFA for each experiment were explored in more depth. If fractal analysis methods such as DFA are to prove useful in welfare assessment it is important to understand more about what it is that they measure. It is particularly important to investigate whether the fractal measures relate to more commonly taken measures of behaviour, e.g. total duration or bout duration. If there is a clear relationship then it may be that fractal analysis is not adding any new or 'hidden' information beyond that normally collected. For each of the experiments the DFA data were compared to the standard measures of behaviour such as duration and frequency.

In the second section, various methodological questions regarding DFA were investigated using the experimental data. Firstly, to validate the analysis methodology, DFA was used to analyse randomly generated sequences. The experiment two data were then used to investigate the effect, on calculated DFA α values, of reducing the total observation time. The data from experiment one, two and three were reanalysed at different sampling frequencies to assess how this affects results.

7-2. Further analysis of experimental data

7-2-1. Analysis

DFA was carried out as previously described in Chapters Four, Five and Six. Where results are presented for correlations, the values given are correlation percentages with the associated P-value in brackets. Where multiple comparisons were made a Bonferroni correction was used to correct for the number of comparisons. The corrected P-value is given following the original one in the format: (original/corrected). As previously, α values for activity and vigilance are referred to as Aα and Vα respectively.

7-2-2. Experiment One: Acute stress in chickens

The values for $A\alpha$ are much lower than those for $V\alpha$ (Fig. 7-1) indicating that activity occurs more randomly than vigilance, which shows a greater level of long-range autocorrelation. As briefly discussed in Chapter Four, there were some problems with applying the DFA analysis to the activity record. In the confined area of their relatively small pen the birds in both experiments spent far less time active than they did vigilant. It was also the case that the recording of activity was less accurate than that of vigilance. Activity was defined as walking with the head up or down and for a bird to be deemed to be walking it had to be seen to take two clear steps. This meant that in the time recorded as inactive the birds could slowly move around, taking only one step at a time. These factors may explain the low $A\alpha$ values: activity may seem more randomly structured because its categorisation relates less directly to the internal motivational state of the animal. It could equally be the case that locomotion is genuinely more randomly organised than vigilance.

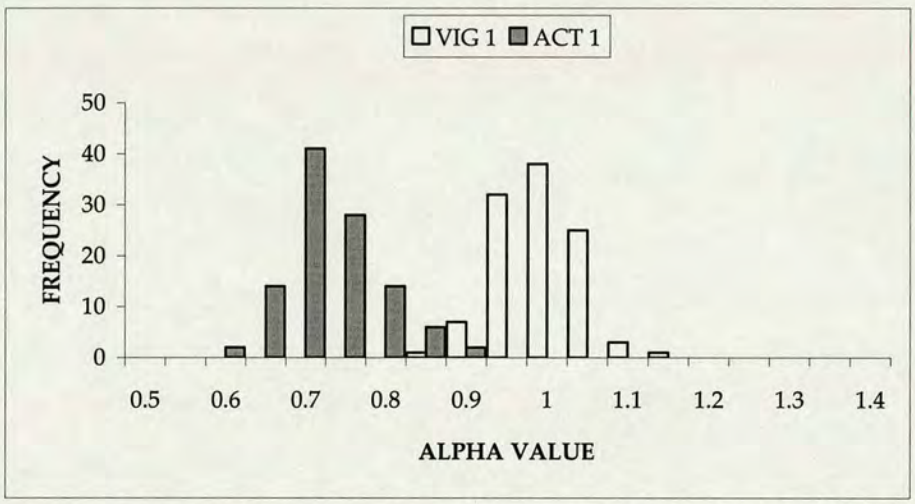


Figure 7-1: Frequency distribution of $V\alpha$ and $A\alpha$ from all observations in experiment one

The $V\alpha$ values were compared with the standard measures of vigilance duration, frequency of vigilant behaviours and mean time spent vigilant (Table 7-1). $V\alpha$ did not correlate at all with the total time spent vigilant or with the mean vigilant duration. However, the $V\alpha$ values were significantly negatively correlated with the

frequency of vigilant behaviours in four out of the six observation situations and in all observations combined.

There were high positive correlations between $A\alpha$ and the mean duration of activity in four out of the six observation situations and when all observations were combined (Table 7-2). $A\alpha$ correlated with the total time spent active in two observation situations and again when all observations were combined. It also correlated with the frequency of activity over all observations and in one out of the six situations.

Table 7-1: Relationship (correlation percentage (p-value)) between $V\alpha$ and standard measures – experiment one

	Frequency		Total Duration		Mean	
Observation						
Home pen one	-38	(0.12)	-10	(0.7)	12	(0.64)
Home pen two	-48	(0.07/0.49)	-10	(0.72)	17	(0.54)
Home pen three	-73	(0.001/0.007)	-8	(0.76)	37	(0.14)
Restraint	-69	(0.002/0.014)	58	(0.15)	54	(0.025/0.175)
Blood sample	-74	(0.001/0.007)	-15	(0.57)	34	(0.2)
Novel arena	-63	(0.001/0.007)	36	(0.09)	36	(0.1)
All	-56	(<0.001/0.007)	-4	(0.71)	22	(0.02/0.14)

Table 7-2: Relationship (correlation percentage (p-value)) between $A\alpha$ and standard measures – experiment one

	Frequency		Total Duration		Mean	
Observation						
Home pen one	53	(0.02/0.14)	64	(0.004/0.028)	67	(0.003/0.021)
Home pen two	8	(0.78)	48	(0.06)	67	(0.004/0.0.028)
Home pen three	52	(0.03/0.21)	58	(0.02/0.14)	24	(0.35)
Restraint	42	(0.09/0.63)	48	(0.05/0.35)	48	(0.05/0.35)
Blood sample	74	(0.001/0.007)	79	(<0.001/0.007)	73	(0.001/0.007)
Novel arena	10	(0.66)	54	(0.008/0.056)	86	(<0.001/0.007)
All	35	(<0.001/0.007)	52	(<0.001/0.007)	52	(<0.001/0.007)

7-2-3. Experiment Two: Chronic-intermittent stress in chickens

The distributions of Aα and Vα in experiment two were broadly similar to those in experiment one (Fig. 7-2). The values are statistically compared in section 7-2-4.

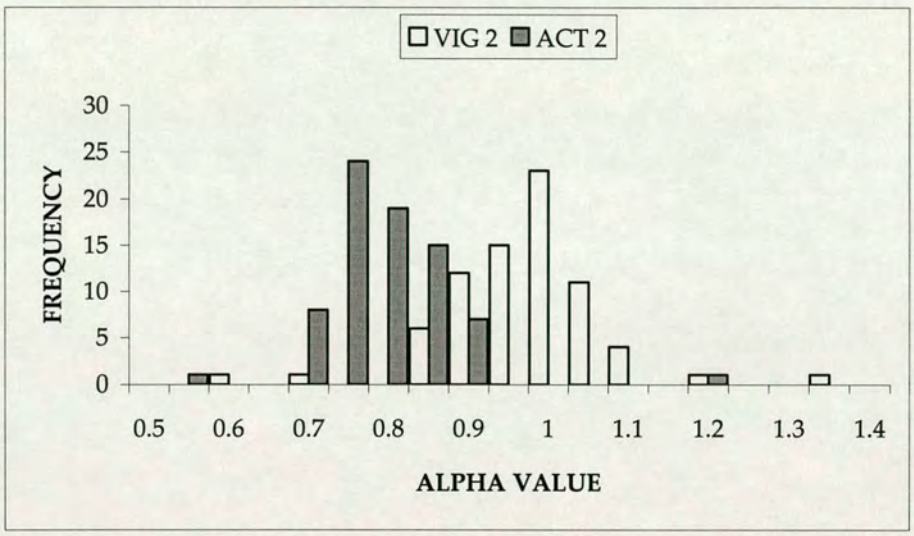


Figure 7-2: Frequency distribution of Vα and Aα from all observations in experiment two

Again, Vα did not correlate with the total time spent vigilant (Table 7-3). There was a strong negative correlation between Vα and the frequency of vigilant states in all four observations, even following correction for multiple comparisons. This means that as the frequency of vigilant states increased the α value decreased indicating a more random pattern. There was also a significant positive correlation between Vα and the mean vigilant duration in three of the observations prior to correction but only one following correction.

There was no relationship between Aα and frequency or total duration of activity (Table 7-4). In the first three observations the data suggest that Aα is positively related to the mean bout length of activity, although following correction for multiple comparisons the relationship is significant in only one observation.

Table 7-3: Relationship (Correlation percentage (p-value)) between Vα and standard measures – experiment two

Observation	Frequency		Total Duration		Mean	
One	-70	(0.001/0.004)	-8	(0.75)	29	(0.24)
Two	-89	(<0.001/0.004)	-15	(0.53)	49	(0.03/0.12)
Three	-81	(<0.001/0.004)	49	(0.04/0.16)	83	(<0.001/0.004)
Four	-85	(<0.001/0.004)	18	(0.49)	50	(0.04/0.16)

Table 7-4: Relationship (Correlation percentage (p-value)) between Aα and standard measures – experiment two

Observation	Frequency		Total Duration		Mean	
One	6	(0.81)	-1	(0.97)	45	(0.054/0.22)
Two	-14	(0.57)	-13	(0.59)	63	(0.004/0.016)
Three	36	(0.15)	39	(0.12)	48	(0.044/0.176)
Four	-15	(0.58)	-18	(0.49)	-8	(0.75)

The Kendall's coefficient of concordance (W) for all values of Vα (i.e. both treatment groups) over the four observations was 0.5 (df=16, Chi-Sq. =32, P=0.01). There was no such significant association for the Aα values (W=0.286, df=16, Chi-Sq.=18.3, P=0.31), indicating that individual birds are consistent across observations in their behavioural complexity for vigilance but not for activity.

From the frequency distribution of bout durations for vigilance and non-vigilance (Fig. 7-3), it can be seen that the majority of durations are very short: 83.9% of vigilant bouts and 70.7% of non-vigilant bout are under 10 seconds in length and 98.5% of both are under one minute. However, the distribution has a very long tail due to a few very infrequent long durations spend in either state. It is these occasional long durations that can mean the DFA cannot be applied to some sequences.

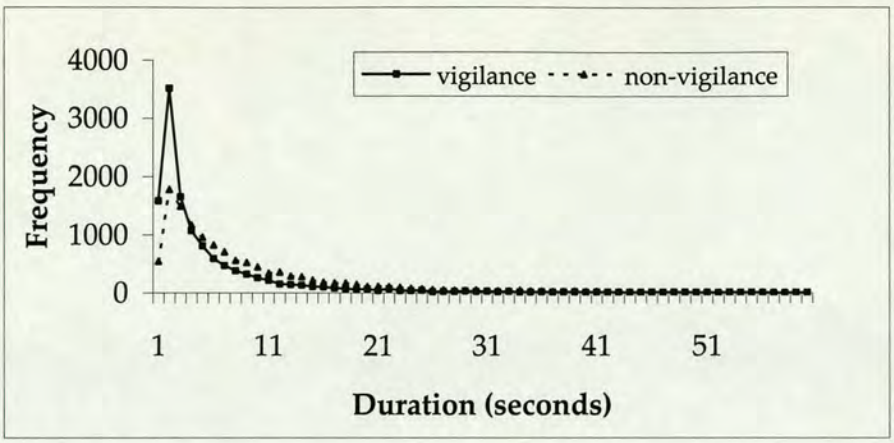


Figure 7-3: Frequency distributions of vigilant and non-vigilant bouts for all observations in experiment two

7-2-4. Comparison of results in experiment one and two

Mean Vα was significantly lower in the second experiment compared to the first (Fig. 7-4: Two-sample t-test, t= 2.19, P=0.03, df= 178), indicating that on average vigilance behaviour was more structured in the birds during experiment one and more random in the second experiment. Conversely, the mean Aα was significantly higher in the second experiment compared to the first (Fig 7-5: t= -6.56, P<0.001, df=178), indicating the opposite: the activity pattern was closer to random in the first experiment and more structured in the second experiment.

Since the two experiment were carried out one year apart there are limits to any comparison between the two. However, the two sets of observations were carried out in the same way, with the same strain, source and rearing of birds and with birds in pairs in the same pens. The main difference between the birds was in their age. In experiment one the birds were between 11 and 17 weeks old, whereas in experiment two the birds were over one year old. So, it is possible to speculate that the difference in behavioural complexity could be age-related. However, this would need to be formally tested.

A comparison of Tables 7-1 and 7-3 shows that the relationship between Vα and the frequency of vigilance, and the lack of a relationship with the total duration of vigilance or the mean vigilant duration, is consistent for both observations. The same results for activity (Tables 7-2 and 7-4) are not at all consistent. This again casts doubt on the usefulness of the Aα measure.

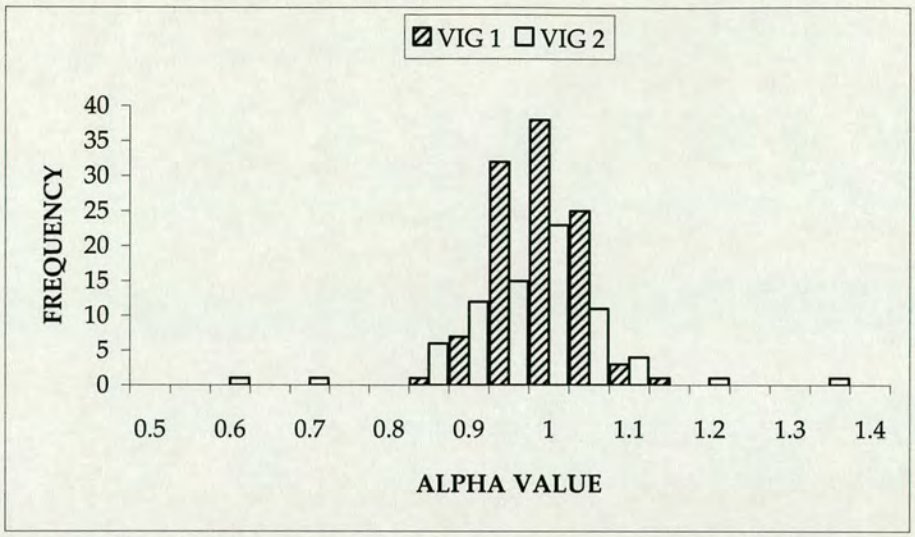


Figure 7-4: Comparison of Vα values in experiments one and two
The Vα values from the first experiment (VIG 1) are in white with black stripes and for the second experiment (VIG 2) are plain white.

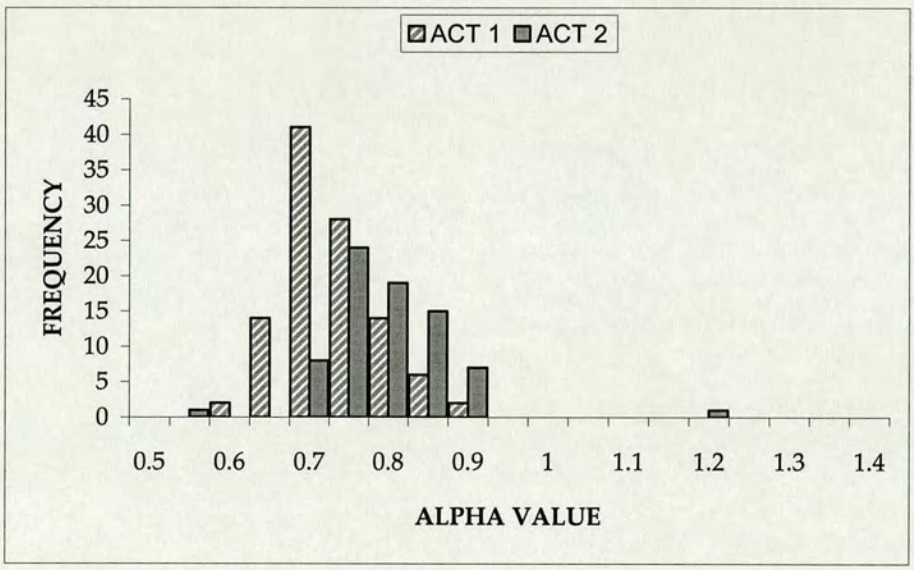


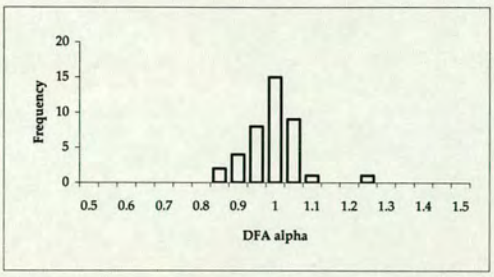
Figure 7-5: Comparison of Aα values in experiment one and experiment two
The Aα values from the first experiment (ACT 1) are in grey with white stripes and for the second experiment (ACT 2) are plain grey.

7-2-5. Experiment Three: Chronic stress in pigs

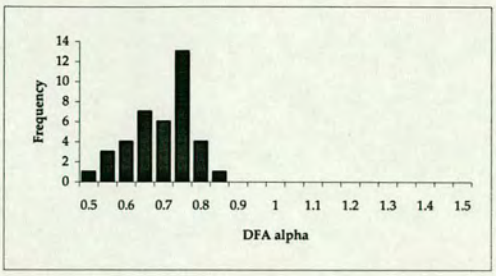
The α values for each of the categories analysed with DFA in experiment three are all highly significantly different from 0.5 (Table 7-5, Fig. 7-6), demonstrating that the behavioural sequences are not randomly structured over time. In each category, α did not correlate with the total number of scans recorded in that category. As with the results from experiments one and two this indicates that the DFA provided extra information about structure beyond that provided by the total amount of behaviour.

Following a Bonferroni correction, the only category where the α values for the two observations (30 days apart; before and after the stressor period) were correlated was feeding (Table 7-5). There were also significant correlations prior to correction in social behaviour, straw-directed behaviour and lateral lying. Interestingly, the feeding α differed between the two observations. Feeding behaviour was significantly more structured in the second observation compared to the first. However, the high correlation between observations indicates that individuals maintained their relative position within the group. What makes these results particularly interesting is that the values for the number of scans spent feeding did not show a correlation or a difference between the two observations (Table 7-6). So the total number of scans recorded as feeding remained constant between observations and there was no individual consistency between observations. In the case of behavioural activity the opposite is true: there is a significant change between observations in the number of counts but not the α values.

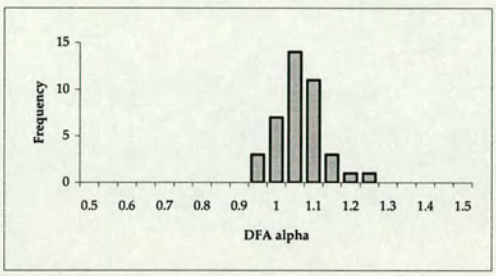
In the case of the total number of scans the values for social behaviour, lateral lying and pig directed behaviour were significantly correlated between observations. There were significant differences between the two observations for the total number of scans observed in behavioural activity, interacting with the environment and straw directed behaviour.



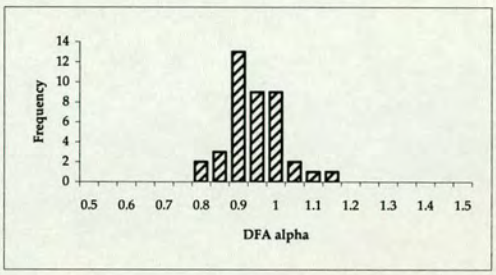
POSTURAL ACTIVITY



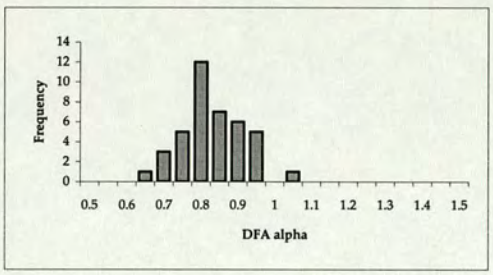
SOCIAL BEHAVIOUR



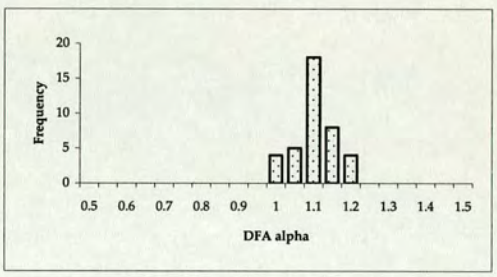
BEHAVIOURAL ACTIVITY



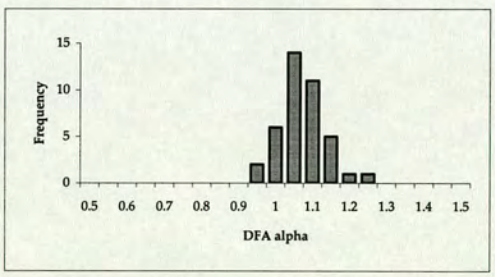
STRAW-DIRECTED BEHAVIOUR



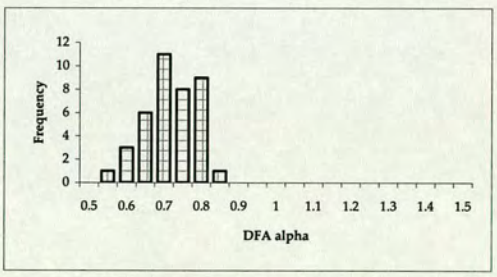
FEEDING BEHAVIOUR



LATERAL LYING



INTERACTING



PIG-DIRECTED BEHAVIOUR

Figure 7-6: Frequency distributions for the *α* value for each of the categories recorded in experiment three

Table 7-5: Statistics for the various α values calculated in experiment three

	Postural Activity	Behavioural activity	Feeding	Interacting	Social	Straw	Lateral lie	Pig
α vs. 0.5 One-sample t-test	t=42.34 P<0.001	t=51.42 P<0.001	t=23.28 P<0.001	t=56.08 P<0.001	t=12.61 P<0.001	t=38.95 P<0.001	t=67.53 P<0.001	t=17.37 P<0.001
Relationship between α and total scans: Corr. % (p-value)	12 (0.47)	6 (0.74)	-11 (0.5)	2 (0.93)	10 (0.57)	1 (0.93)	8 (0.61)	1 (0.98)
Relationship between α for the two observations: Corr. % (p-value)	38 (0.1)	33 (0.16)	69 (0.001 /0.008)	32 (0.17)	54 (0.02/0.16)	44 (0.05/0.4)	54 (0.02/0.16)	39 (0.1)
Mean difference between α for the two observations	-0.03	0.03	-0.06	0.02	-0.04	0.05	0.01	-0.01
α Observation one vs. two One-sample t-test	t=-1.76 P=0.09	t=1.48 P=0.16	t=-4.41 P<0.001 /0.008	t=1.27 P=0.22	t=-2.09 P=0.05 /0.4	t=3.33 P=0.004 /0.032	t=0.93 P=0.36	t=-0.59 P=0.56

First observation value minus second observation value

The fact that there was no correlation between α and the total number of scans within each category does not mean that this relationship does not exist between categories. Indeed, when the mean α value for each category was plotted against the mean number of scans in that category, it was apparent that there was a significant positive correlation (linear: r=0.83, P=0.005, log-linear (Fig. 7-7): r=0.98, P<0.001), indicating that less common behavioural categories are recorded as having a more random structure, even if within a behavioural category there is no relationship between occurrence and structure. Taking the log of the average number of scans improves the fit because there is one category (lateral lying) where the average number of scans was much larger than the other categories.

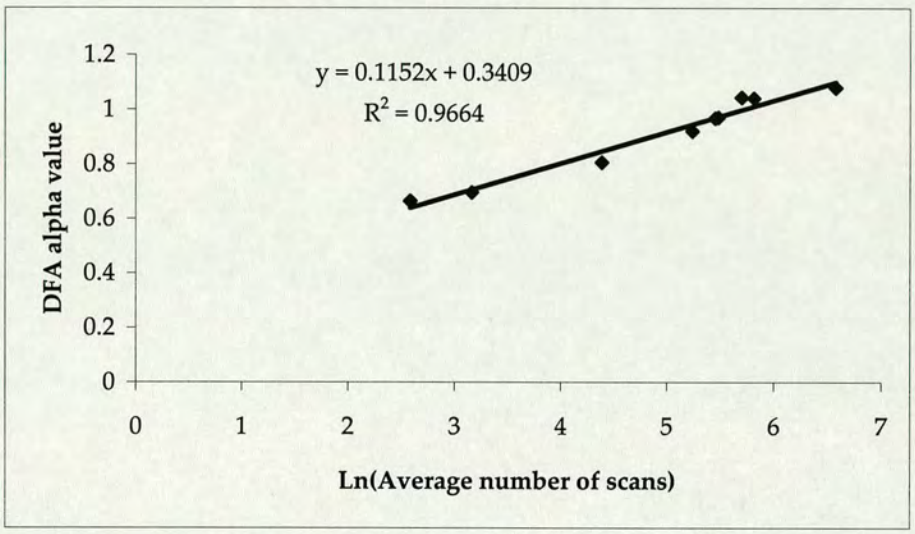


Figure 7-7: Average number of scans (log) plotted against average α value for each behavioural category.

Table 7-6: Relationship (Correlation percentage (p-value)) and differences between the numbers of scans recorded in different categories in the two observations

	Postural Activity	Behavioural activity	Feeding	Interacting	Social	Straw	Lateral lie	Pig
Corr. % (P-value)	18 (0.45)	-6 (0.79)	17 (0.47)	-5 (0.84)	50 (0.026 /0.21)	28 (0.24)	64 (0.002 /0.016)	73 (<0.001 /0.008)
Mean Diff.[1]	17.4	59.9	-0.35	44.6	0.4	46.7	52.9	1.35
One-sample t-test	t=1.21 P=0.24	t=3.22 P=0.004/ 0.032	t=-0.41 P=0.69	t=2.64 P=0.016/ 0.13	t=0.18 P=0.86	t=3.64 P=0.002 /0.016	t=1.92 P=0.07	t=0.56 P=0.58

[1]First observation value minus second observation value

7-3. Analysis of DFA methodology

7-3-1. Analysis of randomised sequences

7-3-1-1. Why analyse randomised sequences?

To validate the program developed to calculate the α exponent it was necessary to run randomly generated values through the program. As explained in section 3-3-2, randomly organised data should produce a mean α value of 0.5.

7-3-1-2. Methods

Random sequences (6144 data points, N=40) were created in Excel (Microsoft Excel 2000). Sequences were generated that represented a 50:50 chance of the behaviour at any time point being A or B. These sequences were analysed at resolutions of half-, one-, two-, four- and eight-seconds. The results for lower resolutions are compared to the results at the half-second resolution, which is the most accurate analysis and is therefore used as a benchmark.

7-3-1-3. Results

There was no significant difference from the expected value of α=0.5 at resolutions of half-, one- or two-seconds (Table 7-7). However, mean α was significantly greater

than 0.5 at resolutions of four- and eight-seconds. The variability in the mean α increases as the resolution is decreased. The one-second resolution results were significantly correlated with the half-second results, but none of the other resolutions correlated with the half-second results. The mean R^2 value was decreased significantly (from the half-second resolution value) at two-, four- and eight-second resolutions. However, the absolute change was very small; even at a resolution of eight seconds the mean R^2 value is 0.986.

These results show that the program works correctly. When entirely random sequences were fed in, it produced values that did not differ from 0.5. However, it is clear that altering the resolution can affect the calculated α value in some cases. Altering the resolution (without a concomitant increase in the observation length) increases sampling variability and decreases the reliability of the results.

Table 7-7: Results of analysis at different resolutions of randomly generated data

Maximum No. of Windows	No. of Data points	Resolution (seconds)	No. of missing sequences	Corr. % with half – sec results (p-value)	Mean α (S.D.)	t-test vs. half-sec results	t-test α vs. 0.5	Mean R^2 (S. D.)	t-test R^2 vs. Half-sec results
1024	6144	0.5	0		0.500 (0.022)		t=0.08 P=0.94	0.996 (0.0025)	
512	3072	1	0	53.3 (<0.001)	0.500 (0.022)	t=0.51 P=0.89	t=-0.06 P=0.95	0.995 (0.0031)	t=1.47 P=0.15
256	1536	2	0	14.1 (0.39)	0.507 (0.026)	t=-1.27 P=0.21	t=1.61 P=0.12	0.993 (0.0054)	t=3.47 P=0.001
128	768	4	0	4.5 (0.78)	0.514 (0.037)	t=-2.04 P=0.048	t=2.37 P=0.023	0.991 (0.006)	t=4.94 P<0.001
64	384	8	0	6.8 (0.68)	0.514 (0.044)	t=-1.83 P=0.076	t=2.04 P=0.049	0.986 (0.009)	t=6.62 P<0.001

N= 40 sequences

7-3-2. Observation time

7-3-2-1. Could the necessary observation time be reduced?

The observation time necessary to carry out a DFA on behavioural data is an important determinant of the practicality of the method. This observation length will naturally differ for different behaviours. The fact that all observations in experiment two were undertaken in stationary conditions (i.e. not following an acute event as in experiment one) allowed an investigation of the effect of shortening observation time to be made. The experiment three data were also analysed at a decreased observation length.

7-3-2-2. Methods

A comparison was made between the results for the full observation time (3072 seconds) and successive reductions in that observation time. Each decrease in duration was based on the decrease in the maximum number of boxes used in the analysis. The number of windows followed the sequence: 2^2, $2^{2.5}$, 2^3, $2^{3.5}$.... 2^{10} (rounded to the nearest integer; 4, 6, 8, 11, ...1024). The full observation length therefore involved a maximum of 1024 windows. The first decrease in observation time was to an observation time of 36m12s (724 windows). Subsequent decreases are shown in Tables 7-10 and 7-11.

Two different methods were used to alter the observation time. In method one the reduced observation times all started from the beginning of the original observation, while in method two the reduced observations were taken from the middle of the observation. This second method was used because of the possibility that the apparent alteration in mean α value for short observation times, seen using method one, represented a genuine alteration in structure at the start of the observation. (Although the birds were not handled prior to the start of the observation, there could well have been a mild disturbance associated with setting up cameras etc that might have affected behaviour).

The question of total observation time also applies when a time sampling method is used. The data collected in experiment three involved one-minute instantaneous behavioural sampling over 24hours. To investigate the effects of decreasing the

observation time the data for the daylight period of the observation (7am to 5pm) were analysed and compared to the full 24h data.

7-3-2-3. Results - Chicken vigilance

The method one analysis (Table 7-10) showed a significant correlation between the $V\alpha$ value for the full observation and all the other analyses for reduced observation times, even down to the shortest observation time of only 2m15s. The correlation coefficient steadily decreased as the observation time was reduced. The mean $V\alpha$ remained roughly constant until sequences were analysed at an observation time of 4m33s and below, when there was an apparent increase. The results from observations of 3m12s and below are significantly different from the full-length observation. The standard deviation also increased with decreasing observation length indicating greater variability in the data. At an observation time of 6m24s the analysis could not be carried out on one sequence and at an observation time of 3m12s another sequence could not be analysed. The reason why these sequences could not be analysed was that the birds did not change their behaviour over these short periods.

The results for method two (Table 7-11) were similar to those for method one. Compared to method one, the correlation with the full analysis decayed more quickly as observation time was reduced and the mean α value significantly differed from the original at an observation length of 6m24s. The increase in α value and greater variability at short observation times were consistent, suggesting that the alteration in α is a genuine consequence of the shortened observations, rather than a result of any 'start-up' effect. Using method two, one sequence could not be analysed at the 9m3s observation length, another could not be analysed at the 4m33s observation length and a total of six could not be analysed at the lowest observation length of 2m15s.

Table 7-10: The effect of reducing observation time on the DFA α value for vigilance behaviour using data recorded in experiment two (METHOD ONE)

No. of Windows	No. of Data points	Observation time	No. of missing sequences	Mean α (S.D.)	Corr. % with full analysis (p-value)	t-test vs. full Analysis
1024	6144	51m 12s	0	0.946 (0.097)	1	
724	4344	36m 12s	0	0.948 (0.1)	93 (<0.001)	t= -0.48 P=0.63
512	3072	25m 36s	0	0.941 (0.1)	77 (<0.001)	t= 0.57 P=0.57
362	2178	18m 9s	0	0.946 (0.11)	71 (<0.001)	t= -0.07 P=0.94
256	1536	12m 48s	0	0.942 (0.13)	63 (<0.001)	t= 0.29 P=0.77
181	1086	9m 3s	0	0.941 (0.13)	59 (<0.001)	t= 0.35 P=0.73
128	768	6m 24s	1	0.942 (0.12)	51 (<0.001)	t= -0.09 P=0.93
91	546	4m 33s	1	0.96 (0.13)	44 (<0.001)	t= -1.32 P=0.19
64	384	3m 12s	2	0.98 (0.14)	46 (<0.001)	t= -2.87 P=0.005
45	270	2m 15s	2	0.99 (0.16)	36 (0.002)	t= -3.02 P=0.004

N= 73 sequences

Table 7-11: The effect of reducing observation time on the DFA α value for vigilance behaviour using data recorded in experiment two (METHOD TWO)

No. of Windows	No. of Data points	Observation time	No. of missing sequences	Mean α (S.D.)	Corr. % with full analysis (p-value)	t-test vs. full Analysis	Mean R^2 (S.D.)
1024	6144	51m 12s	0	0.946 (0.097)	100		0.994 (0.0046)
724	4344	36m 12s	0	0.945 (0.102)	86 (<0.001)	t= 0.21 P=0.84	0.991 (0.009)
512	3072	25m 36s	0	0.940 (0.104)	67 (<0.001)	t= 0.65 P=0.52	0.989 (0.009)
362	2178	18m 9s	0	0.949 (0.104)	67 (<0.001)	t= -0.35 P=0.72	0.987 (0.010)
256	1536	12m 48s	0	0.944 (0.114)	65 (<0.001)	t= 0.16 P=0.87	0.985 (0.010)
181	1086	9m 3s	1	0.944 (0.113)	36 (0.002)	t= 0.05 P=0.96	0.982 (0.012)
128	768	6m 24s	1	0.980 (0.127)	47 (<0.001)	t= -2.89 P=0.005	0.983 (0.011)
91	546	4m 33s	2	0.995 (0.166)	36 (0.002)	t= -2.99 P=0.004	0.979 (0.015)
64	384	3m 12s	2	1.003 (0.160)	23 (0.05)	t= -3.24 P=0.002	0.976 (0.015)
45	270	2m 15s	6	1.044 (0.187)	20 (0.1)	t= -4.73 P<0.0010	0.969 (0.030)

N= 73 sequences

7-3-2-4. Results - Pig behaviour

The daytime α value was highly correlated with the value over the full 24h for all categories (Table 7-12). In each case the daytime values were significantly higher than the full 24hour values – indicating a greater degree of long-range autocorrelation, as expected for a shorter time series.

In Chapter Six it was shown that the pattern of postural activity over 24hours differed between test and control pigs following a stressor treatment but not before (although there was a trend for a difference before as well). To investigate the effect of observation length further the day only DFA values for each of the behavioural categories were compared between treatments (Table 7-13). This analysis showed that for the pattern of postural activity during daytime there was a significant difference between test and control pigs before and after the stressor treatment. The difference following the stressor treatment was significant even following a Bonferroni correction, while the difference before was not significant following Bonferroni correction.

Table 7-12: Reanalysis of experiment three data using daytime data only

Behavioural category	Full Mean α (S. D.)	Day Mean α (S. D.)	Day Corr. % with 24h (p-value)	Day t-test vs. 24h
Postural activity	0.968 (0.070)	1.053 (0.076)	88 (<0.001)	t=-14.86 P<0.001
Behavioural activity	1.040 (0.067)	1.125 (0.076)	80.2 (<0.001)	t=-11.64 P<0.001
Feeding	0.806 (0.083)	0.858 (0.095)	90.5 (<0.001)	t=-8.13 P<0.001
Interacting	1.044 (0.061)	1.122 (0.070)	79.9 (<0.001)	t=-11.62 P=<0.001
Lateral lie	1.079 (0.054)	1.157 (0.073)	53.4 (<0.001)	t=-7.51 P<0.001
Pig	0.695 (0.070)	0.714 (0.103)	90.9 (<0.001)	t=-3.18 P=0.003
Social	0.663 (0.082)	0.692 (0.101), n=39	92.6 (<0.001)	t=-4.44 P<0.001
Straw	0.920 (0.068)	0.957 (0.085)	85.9 (<0.001)	t=-5.49 P<0.001

Table 7-13: DFA of experiment three data – day time only

DFA Category	Before			After			Before vs. After	
	Control	Test	Statistics	Control	Test	Statistics	Control	Test
Postural Activity	1.004 (0.087)	1.065 (0.061)	t=-2.74 P=0.023	1.046 (0.061)	1.097 (0.071)	t=-4.72 P=0.001	t=-1.48 P=0.17	t=-1.14 P=0.28
Behavioural Activity	1.117 (0.077)	1.157 (0.089)	t=-1.67 P=0.13	1.101 (0.069)	1.123 (0.067)	t=-0.71 P=0.49	t=0.74 P=0.48	t=1.16 P=0.27
Feed	0.893 (0.108)	0.882 (0.122)	t=0.37 P=0.72	0.936 (0.1490)	0.978 (0.146)	t=-0.54 P=0.6	t=-0.89 P=0.4	t=-2.16 P=0.06
Interact	1.115 (0.065)	1.147 (0.079)	t=-1.3 P=0.23	1.102 (0.074)	1.126 (0.066)	t=-0.73 P=0.49	t=0.65 P=0.53	t=0.74 P=0.48
Lateral	1.147 (0.075)	1.151 (0.079)	t=-0.12 P=0.91	1.1726 (0.071)	1.155 (0.076)	t=0.58 P=0.58	t=-1.06 P=0.32	t=-0.1 P=0.92
Pig	0.717 (0.129)	0.690 (0.076)	t=0.58 P=0.57	0.706 (0.106)	0.744 (0.103)	t=-1.95 P=0.08	t=0.27 P=0.79	t=-1.51 P=0.17
Social	0.667 (0.117)	0.669 (0.093)	t=-0.11 P=0.92	0.730 (0.076)	0.730 (0.089)	t=-0.01 P=0.99	t=-1.22 P=0.25	t=-1.98 P=0.08
Straw	0.986 (0.066)	0.989 (0.100)	t=-0.11 P=0.92	0.931 (0.082)	0.924 (0.076)	t=0.19 P=0.85	t=2.08 P=0.07	t=3.07 P=0.013

7-3-3. Sampling frequency

7-3-3-1. How does sampling frequency affect DFA results?

Another aspect of the analysis methodology that could affect the results is the resolution, or sampling frequency, of the analysis. Since, for some behaviours, the accuracy of a human observer may not be great enough to adequately record its pattern of occurrence, the ultimate upper limit on resolution is the accuracy of the human observer. Pecking had been recorded in an early pilot study during this project but it was felt that the time scale of pecking was too fine for the observer to accurately pick up any variation that might be suitable for fractal analysis. Pecking can be recorded automatically using a 'peck-o-meter' (Bessei et al. 1997; Hocking et al. 1999) and this could provide the necessary level of accuracy. Other automated collection devices might be suitable for analysing basic activity patterns or feeding with a high degree of accuracy (e.g. Nielsen et al. 1995; Noldus et al. 2001), but more complex behaviours still require a human observer.

To prevent the problem of aliasing (where infrequent sampling smoothes out small scale, high frequency fluctuations) it is recommended that data should "be sampled at more than twice the highest frequency of interest", a frequency known as the Nyquist frequency (Gershenfield 1999). In terms of behaviour, initially the necessary sampling frequency will relate to the shortest time that an animal might remain in a particular behavioural state. With chicken vigilance, some fluctuations do occur on a scale less than one second, e.g. when a foraging or preening bird raises its head and then immediately returns to the previous behaviour. These brief changes may or may not be important and comparing results at different resolutions will help elucidate this. However, below one second the error due to reaction time and interpretation by the observer will be relatively large. So although a sampling frequency of half a second might be strictly speaking less than the Nyquist frequency it is not meaningful to go to a higher frequency. In their study of chimpanzee social behaviour Alados and Huffman (2000) used a sampling interval of five seconds. Since fluctuations between social and non-social behaviour are likely to occur less frequently this should be an adequate level of sampling in this case. Indeed the authors note that durations of social behaviour were never less than this.

Although continuous focal sampling was used in the experiments described in Chapters Four and Five, instantaneous sampling could be more practical for future use, so an assessment of the effects of altering resolution may inform what a suitable sampling interval might be.

7-3-3-2. Methods

Both the experiment one and two results were re-analysed at resolutions of one-, two-, four- and eight-seconds and the results compared to the half-second resolution used in the original analysis. Each successive decrease in resolution (i.e. less frequent sampling) represents a halving of the amount of information obtained from the observation. (Note; a similar reduction in the amount of information gathered is achieved by reducing total observation and maintaining resolution in Tables 7-10 and 7-11). Although an eight-second sampling interval would not be high enough to allow a practical scan sampling protocol to be used on a group of animals the fact that there was only 3072 seconds worth of behavioural observation to work with limits the extent to which the sampling frequency can be altered. Any resolution below eight seconds would not provide enough data points to accurately run the analysis. These data do still allow the effect of altering resolution to be analysed to some extent though.

7-3-3-3. Results - Effect of altering resolution on Vα

The results for the altered sampling frequency analysis (Tables 7-14 and 7-15) are very clear and consistent for both data sets. As the sampling frequency decreased, Vα significantly decreased (i.e. the behavioural sequence is recorded as being closer to random) but remained highly correlated with the results from the highest sampling frequency. The standard deviation also increased as sampling frequency was decreased. The mean R^2 value (representing the goodness of fit in the log-log plots) also decreased slightly (but significantly at two-, four- and eight-seconds compared to half-second) and became more variable as sampling frequency increased.

Table 7-14: The effect of altering observation resolution on the α value for vigilance behaviour using data recorded in experiment one

Maximum No. of Windows	No. of Data points	Resolution (seconds)	Observation time	No. of missing sequences	Corr. % with full analysis (p-value)	Mean α (S. D.)	t-test vs. full Analysis	Mean R^2 (S.D.)
1024	6144	0.5	3072	0	1	0.971 (0.050)		0.994 (0.0034)
512	3072	1	3072	0	99 (<0.001)	0.937 (0.058)	t=31.79 P<0.001	0.995 (0.0034)
256	1536	2	3072	0	95.5 (<0.001)	0.907 (0.067)	t=27.69 P<0.001	0.993 (0.0047)
128	768	4	3072	0	90.3 (<0.001)	0.877 (0.080)	t=24.48 P<0.001	0.990 (0.0069)
64	384	8	3072	0	82.7 (<0.001)	0.832 (0.096)	t=23.96 P<0.001	0.985 (0.010)

N = 114 sequences

Table 7-15: The effect of altering observation resolution on the α value for vigilance behaviour using data recorded in experiment two

Maximum No. of Windows	No. of Data points	Resolution (seconds)	Observation time	No. of missing sequences	Corr. % with full analysis (p-value)	Mean α (S. D.)	t-test vs. Full Analysis	Mean R (S. D.)
1024	6144	0.5	3072	0	1	0.946 (0.097)		0.994 (0.0046)
512	3072	1	3072	0	99.3 (<0.001)	0.913 (0.106)	t=18.35, P<0.001	0.994 (0.0047)
256	1536	2	3072	0	97 (<0.001)	0.884 (0.117)	t=16.75, P<0.001	0.992 (0.0064)
128	768	4	3072	0	94 (<0.001)	0.846 (0.129)	t=16.55, P<0.001	0.990 (0.008)
64	384	8	3072	0	89 (<0.001)	0.807 (0.143)	t=16.23, P<0.001	0.983 (0.0151)

N= 73 sequences

7-3-3-4. Results - Treatment comparisons with altered resolution

Table 7-16 shows the effect of altering the sampling resolution on the behavioural treatment differences identified in experiment one (Chapter Four). The original result, with a resolution of half a second, showed that the vigilance behaviour observed in the novel arena and following restraint and blood sampling was more random than that in the home pen. For the novel arena observation this result remained significant at the one- and two-second resolutions and is only just over significant at the four-second resolution. However, at the eight-second resolution the difference between the novel arena and home pen observations was no longer significant. There was no significant difference between the observations following blood sampling alone and the undisturbed observations, at any resolution. For the comparison between the restraint and blood sampling observations and the home pen observation the P-value of the one-sample t-test increased at the increased sampling resolutions. The one-, four- and eight-second resolutions produced a value over 5% (although the one second resolution analysis was only just over). The absolute magnitude difference between either the novel arena or restraint observation and the home pen observations remained fairly constant or increased as resolution was altered. However, the variability in the data increased and this affects the significant level.

Table 7-16: Effect of altering resolution on treatment differences in Vα (Experiment one)

	Resolution (seconds)				
	Half	**One**	**Two**	**Four**	**Eight**
[1]**Home pen: Mean (S. D.),**	0.985 (0.042)	0.953 (0.049)	0.928 (0.058)	0.896 (0.068)	0.852 (0.080)
[2]**NA – HP: Mean (S.D.)**	-0.0385 (0.042)	-0.0384 (0.049)	-0.0375 (0.062)	-0.0328 (0.082)	-0.0323 (0.12)
One Sample T-Test	t=-4.44, P<0.001	t=-3.78 P=0.001	t=-2.92 P=0.008	t=-1.92 P=0.068	t=-1.29 P=0.21
[3]**BS-HP: Mean (S.D.)**	-0.0087 (0.037)	-0.0144 (0.045)	-0.0223 (0.055)	-0.0237 (0.070)	-0.0217 (0.103)
One Sample T-Test	t=-0.94, P=0.36	t=-1.27 P=0.22	t=-1.63 P=0.12	t=-1.35 P=0.20	t=-0.85 P=0.41
[4]**RBS-HP:Mean (S.D.)**	-0.0246 (0.042)	-0.0256 (0.050)	-0.0329 (0.061)	-0.0377 (0.0765)	-0.0406 (0.095)
One Sample T-Test	t=-2.42, P=0.028	t=-2.1 P=0.052	t=-2.23 P=0.041	t=-2.03 P=0.059	t=-1.76 P=0.097

1: One value was calculated for each pen, based on one, two or three observations. N=24

2: NA= novel arena. N=23

3: BS = blood sample. N=16

4: RBS = restraint and blood sample. N=17

7-3-3-5. Results - Effect of altering resolution on DFA of pig behaviour

The experiment three data were re-analysed at a sampling interval of two and three minutes and compared to the original one-minute analysis (Table 7-17). There were significant positive correlations in all behavioural categories between the original α values and the two- and three-minute resolutions. Apart from the relatively infrequent categories of pig-directed behaviour and social behaviour the correlations were very high. This indicates that moving from a one-minute sampling frequency to a two- or three- minute sampling frequency caused minimal loss of information. What is also very clear, however, is that the decreased sampling frequency caused a large bias towards a lower α value. The behavioural categories appear more random when they are observed less frequently.

Table 7-17: The effect of altering observation resolution on the α value for all behavioural categories (experiment three)

Behavioural category	One-minute Mean α (S. D.)	Two-minute Mean α (S. D.)	Three-minute Mean α (S. D.)	Corr. % (p-value) One vs. two	Corr. % (p-value) One vs. three	Corr. % (p-value) Two vs. three	t-test One vs. two	t-test One vs. three	t-test Two vs. three
Postural activity	0.968 (0.070)	0.894 (0.076)	0.823 (0.085)	96.4 (<0.001)	84.2 (<0.001)	91 (<0.001)	t=22.68 P<0.001	t=19.94 P<0.001	t=12.77 P<0.001
Behavioural activity	1.040 (0.067)	0.985 (0.079)	0.921 (0.100)	96.6 (<0.001)	92 (<0.001)	91.9 (<0.001)	t=15.41 P<0.001	t=15.83 P<0.001	t=9.56 P<0.001
Feeding	0.806 (0.083)	0.721 (0.091)	0.643 (0.093)	94.9 (<0.001)	85.3 (<0.001)	84.7 (<0.001)	t=18.53 P<0.001	t=21.18 P<0.001	t=9.71 P<0.001
Interacting	1.044 (0.061)	0.985 (0.078)	0.919 (0.099)	96 (<0.001)	91.2 (<0.001)	92.2 (<0.001)	t=14.33 P<0.001	t=15.86 P<0.001	t=10.39 P<0.001
Lateral lie	1.079 (0.054)	1.008 (0.064)	0.932 (0.084)	97.2 (<0.001)	84.5 (<0.001)	90.1 (<0.001)	t=25.24 P<0.001	t=21.34 P<0.001	t=13.46 P<0.001
Pig	0.695 (0.070)	0.638 (0.077)	0.612 (0.084)	78.8 (<0.001)	33.1 (0.04)	46.5 (0.002)	t=7.41 P<0.001	t=5.64 P<0.001	t=1.83 P=0.074
Social	0.663 (0.082)	0.604 (0.071)	0.579 (0.076)	68.2 (<0.001)	43.5 (0.007)	59.9 (<0.001)	t=5.92 P<0.001	t=6.42 P<0.001	t=2.47 P=0.018
Straw	0.920 (0.068)	0.871 (0.082)	0.830 (0.103)	96.2 (<0.001)	90.9 (<0.001)	92.2 (<0.001)	t=12.36 P<0.001	t=11.37 P<0.001	t=6.2 P<0.001

7-4. Discussion

7-4-1. Overview

Several conclusions regarding the DFA methodology can be drawn from the analyses presented in this chapter. In all three data sets it is clear that the α value calculated by DFA did not relate to the total duration of the relevant behaviour. The analysis in Chapter Four showed that the α measure can alter when total duration does not. Although the Vα values are negatively correlated to the frequency of vigilant behaviours, the Vα measures identified differences between treatments in the first experiment (Chapter Four) when the frequency of vigilant behaviours did not significantly differ between treatments. The negative correlation between Vα and frequency of vigilant behaviours means that animals showing more frequent vigilance have a more random pattern of vigilance. The Vα measure incorporates information about frequency of vigilant behaviours and the duration of vigilance, yet neither of these two measures, on their own, provides any information about sequence structure. Any given frequency of vigilant behaviours could be structured in a perfectly regular way or in an entirely random way.

These results suggest that the α value does indeed provide extra information about behavioural organisation, beyond that provided by simpler analyses. However, the experiment three data shows that there can be a relationship, over different behaviours, between the occurrence of individual behaviours and their relative position on the DFA scale with less frequent behaviours occurring more randomly.

One interesting result from experiment three was that the DFA description of feeding revealed both changes and consistency between the two observations that were not apparent in the total number of scans recorded as feeding. It might be the case that the consistency of feeding complexity represents a behavioural trait of individual animals, i.e. some animals are consistently more structured in their behaviour than others. However, the fact that the category is feeding suggests that the difference could result from the effect of competition for spaces at the feeder. Although the feeders provided to the pigs were large enough that all three pigs could theoretically feed at the same time, it was extremely rare to see all three feeding at once. It is presumed that dominant animals choose to feed at times

according to their hunger motivation. Subordinate pigs will also feed when hungry but may be displaced if a dominant comes along. Feeding is socially facilitated and synchronised (Morgan et al. 1999), so when a pig sees another pig starting to feed it will also feed. This means that when a subordinate starts to feed it might well trigger a dominant to want access to the feeder and displace the smaller animal. This could lead to different patterns of feeding behaviour according to the social relationships between the three pigs.

Individual pigs in groups can reach the same food intake though greatly differing feeding patterns (e.g. different combinations of feed intake per visit and number of visits) (Nielsen et al. 1995). Individually housed pigs will take many small meals, while group housed pigs partially adjust and take fewer but larger meals. However, Nielsen and associates (1995) did not find any correlations between feeding parameters and social rank. Dominance relationships could well remain consistent over the period between the two observations, so it could be this that causes a consistency in the complexity of feeding behaviour. Rather than referring to a behavioural trait the consistency may reflect a stable behavioural state.

7-4-2. Observation length and resolution

Continuous focal sampling for nearly one hour, as done in experiments one and two, is a very labour intensive job, particularly when the behaviour of interest is changing on a short time-scale, such as is the case with vigilance. To make DFA more practical it would either be possible to reduce the total observation time of a focal sample, or switch to a scan sampling method that would allow more birds to be recorded in a single observation period.

To decrease the sampling frequency it might be necessary to increase observation length, but a time sampling method would allow many individuals to be assessed during one observation. The decision as to whether to go for a shorter focal observation or a longer period of scan sampling will need to be made on a case by case basis, depending on species, behaviour of interest and practicality (e.g. scan sampling requires that individuals be quickly identifiable).

The analyses presented in this chapter suggest a tendency for vigilance behaviour in chickens to be recorded as more structured (higher α) when observation time was greatly reduced. Conversely, α decreased (implying a more

random structure) when vigilance behaviour was sampled less frequency. These two findings match intuitive expectation as to the effects of the two alterations. Since α relates to the autocorrelation structure of the data, when shorter data sets are used the time series will appear more structured because the degree of autocorrelation is being assessed over a shorter period. When sampling frequency is reduced the data appears more random because the data points being analysed are not truly consecutive. Put simply: if a bird is showing behaviour 'x' at a given time point, there is more chance of it still showing behaviour 'x' half a second later than eight seconds later. The analysis here showed that even at an eight-second interval there was still some appreciable structure. However, at some higher interval the sequence would become utterly random.

The necessary observation length and/or sampling frequency will depend on the bout length distribution of the behaviour in question. In the case of chicken vigilance behaviour it can be seen that the majority of durations are less than ten seconds in length (Fig. 7-3). As the sampling interval increases from half a second a large amount of information may be lost about the structure of the behavioural sequence. For vigilance in chickens it is not therefore appropriate to move to a time sampling method. The duration of focal observation could, however, be reduced from 3072 seconds without too much information being lost. Observation times of shorter than 3072s are perfectly valid. Although, as observation time decreases the sequences appear more structured, if the DFA method is to prove useful the absolute α values are less important than any relative change due to stress. The decreasing correlation between the full-length observation and the shorter observation times does not really indicate that the Vα values for these observations are wrong – just different. It is the case, however, that longer observations are generally superior. Treatment differences might reveal themselves on different scales – so if short observations are used it is potentially the case that differences might not be identified. Equally, and especially in the case of acute stress situations, lengthy observations might dilute treatment differences if the change in behaviour is only transient.

Observation length can affect the identification of treatment effects. When Alados and colleagues (1996) applied spectral analysis to sequences of feeding and vigilance behaviour in Ibex they found a significant difference between parasitised

and healthy Ibex using an observation length of 1024s but no difference was found at an observation length of 2048s.

The tail of the bout length frequency distribution of the behaviour in question is the other important parameter to take into account when deciding suitable observation times. The observation time cannot be less than the maximum duration or inter-behaviour-interval of the behaviour in question. Indeed the observation time should ideally be several times the maximum duration or interval.

The longer behavioural durations seen in pigs mean that scan sampling is appropriate for them. The decision has to whether scan sampling is valid and, if so, at what frequency should ideally be made on the basis of the frequency distribution of behavioural durations. Ideally, the sampling interval should be less than the smallest bout length seen and continuous observation will naturally provide the best record. However, for behaviours with long durations, continuous observation might be redundant if a time sampling method described the sequence just as well (within certain bounds of acceptable error). For any behavioural sequence, when continuous focal sampling is abandoned in favour of scan time samples there is a inevitable decrease in the amount of information gained and there will come a point, as sampling interval increases, where the data collected are not a fair representation of the behavioural pattern seen.

Although no formal analysis of bout duration distribution was made it is felt that for the activity (behavioural, postural and lateral lie) categories and for feeding the one-minute sampling frequency gave a reasonable record of the behaviour. For the social or pig categories the record was probably far less accurate. The 'interacting' and 'straw' categories are probably intermediate. Some indication of the appropriateness of scan sampling for various behaviours can be gleaned from the literature. Nielsen and co-workers (1995) kept young pigs, of the same breed as used in experiment three, in groups of various sizes and monitored their feeding behaviour over several weeks with an automated recording system. The average duration of each feeding visit was 4.28 minutes for pigs kept in groups of five (the closest to the group size used here), with the average total time spent feeding each day being 63.3 minutes. De Haer & Merks (1992) kept growing pigs in groups of eight and found a mean feeding time of 6.9min and a daily total feeding time of 63.5min. Hyun and co-workers (1997) found an average feeder occupation time per

visit of between six and seven minutes, depending on sex and diet and a mean total time feeding per day of between 73 and 78 minutes in group-housed (five per pen) pigs. In experiment three, the mean number of scans where the pig was recorded in the feeder was around 70. The values from the literature suggest that this is roughly what should be expected, so it seems likely that few instances of feeding were missed. (This was also my subjective impression when viewing the videotape). Scan sampling may not provide the most accurate record of the fine scale details of within meal intervals, but will be accurate for the pattern of meals and between meal intervals.

As far as activity (whether postural or behavioural) is concerned there is less data on the fine detail, but probably less need for fine detail analysis as pigs are commonly found to spend around 80% of their time inactive (Signoret et al. 1975; Gonyou et al. 1992; Curtis et al. 2001). Most assessments of time budgets are themselves based on scan sampling, so the mean and distribution of durations spent active, inactive, or in particular behaviours such as rooting or social behaviour, is not known. However, the mean time active will be greater than that for feeding and the mean time inactive will be much larger still.

For the pig behaviour data it was seen that the effect of increasing the sampling interval was to greatly lower the α value for each category. As described above, as the sampling interval increases the degree of relationship between successive data points decreases. However the α values recorded at a interval of 3 minutes were closely correlated with the values recorded at one minute, for the most commonly occurring behaviours. There would apparently be no great loss of information if behaviour was only observed over the daylight period and a sampling frequency of one-minute was maintained. It would be useful to investigate a shorter and more detailed assessment of pig behaviour in the future. This would need to be centred on peak time of activity (e.g. the first two hours following lights on in the morning) but might reveal a different sort of information to that provided by scan sampling over longer periods.

Since the behaviours that may be most useful for fractal analysis are activity and feeding, automated data collection might be the way forward. Other behaviours occur infrequently and may be of less use for fractal analysis. It might be possible to

target active times and do a more detailed focal sample on these behaviours (e.g. interacting, social behaviour etc).

In terms of activity there was a difference in fractal dimension between postural activity and behavioural activity; although the two do greatly overlap there still remains periods when the animals may be classified as being posturally inactive (ventral or lateral lying) and be behaviourally active (most commonly chewing straw, occasionally nosing other pig, or being alert). Behavioural activity might be harder to assess using certain automated methods, e.g. methods based on movement such as ethovision. However, biotelemetry devices might allow a far more accurate assessment of periods of activity versus rest through characterisation of EEG patterns (e.g. Langford et al. 2002). Systems that recognise movement patterns could be easily applied to postural activity.

The analysis of feeding patterns for individual pigs is relatively common. Various automated recording systems exist (e.g. Nielsen et al. 1995; Hyun et al. 1997). These systems have a transponder system where entry into an enclosed feeder by individual pigs and the amount eaten and time spent at the feeder is automatically logged and would supply data that is very suitable for this type of analysis.

7-4-3. Suitability of behaviours for DFA

When picking suitable behaviours to apply the analysis on, it is important that those behaviours can be consistently expected to occur in any given observation period. If some sequences cannot be analysed because the behaviour does not occur it is best to avoid using that behaviour, rather than just discarding the particular sequences. (This is particularly true when using the analysis to identify treatment-induced changes in behaviour). This naturally limits the number of different behaviours that can be analysed. However, discarding some sequences and not others is incorrect, especially when the results are being used for treatment comparisons. In Alados and Weber (1999) sequences of behaviour were not included in the analysis if the total time spent in the behaviour being analysed was less than 3 seconds out of a total observation time of five minutes (3000 data points at a resolution of 0.1s). The authors note:

> "Personal observations revealed that when the activity recorded was scarcely represented, i.e., <1% of the entire sequence, the scaling exponent varied depending

on the sequential ordering, which may be altered by modifying the starting time of the recording"

Also, in Alados and Weber (1999) sequences where the behaviour analysed occurred continuously for the whole observation were counted in the analysis as having an α value of 1.5. This again seems incorrect, particularly in light of the fact that the treatment had a significant affect on the total time spent in particular behaviours. This means that the results for fractal complexity could be biased by the few sequences that were erroneously recorded as having an α of 1.5.

In two of Alados' studies (Alados et al. 1996; Alados & Huffman 2000) the sequences of behaviour used for fractal analysis were selected on the basis of the presence of the behaviours of interest. In Alados and colleagues' (1996) study, observations were deliberately limited to times when the ibex were feeding and in Alados and Huffman (2000) sequences were not analysed if the chimpanzee in question was resting at the start or finish. This practice of picking and choosing, which behavioural observations are used and which are not, is obviously far from ideal. The analysis therefore needs to be limited to categories that are reliably seen.

7-5. Conclusions

There are often practical limitations on how much behavioural data can be collected. Decreasing observation time and decreasing resolution represent alternative ways of reducing the amount of behavioural information collected. For vigilance behaviour in chickens, it is concluded that using focal sampling and reducing observation time is preferable to abandoning focal sampling for a scan sampling methodology and decreased resolution. This is because the increase in $V\alpha$, seen as observation length is decreased, is a genuine reflection of the structure of the behaviour, whereas the decreased α values seen as resolution decreased reflect increasing inaccuracy of the method.

For behaviours such as general activity and feeding in pigs a shorter total observation duration and the continued use of one-minute sampling is preferable.

Chapter Eight

Discussion and conclusions

8-1. Introduction

The overall aim of this thesis was to investigate the use of a novel behavioural analysis methodology, fractal analysis, in animal welfare assessment. Previous applications of various fractal techniques in medical physiology and in behavioural studies suggested that they could be used to reveal aspects of the temporal organisation of a process that might otherwise be missed. In many of these studies these parameters varied along with some aspect of health status. One particular fractal analysis method, Detrended Fluctuation Analysis (DFA), was chosen for use. DFA was chosen for several reasons: it is mathematically relatively simple, it had been successfully applied to behavioural observations previously and had also been the subject of numerous mathematical explorations, so was reasonably well understood. The measure produced by DFA describes behavioural patterns along a scale from complete randomness to increasing degrees of long-range autocorrelation. It is easy to apply and is not affected by non-stationarities in the data sequence. Since non-stationarity might be seen as a defining property of animal behaviour, this method is particularly well suited to behavioural data (as opposed to some other methods such as spectral analysis, which can be affected by non-stationarities).

In the course of the project, three experiments were carried out on the two most intensively housed common farm animals, chickens and pigs. These experiments provided a large data set with which to investigate the application of DFA to behavioural data. An experimental approach was taken in which standardised stressors were applied and the resulting behavioural and physiological responses of the animals measured. As far as possible many different measures, both behavioural and physiological, were taken to allow comparison of the DFA results with more standard behavioural, endocrine and immunological indicators of stress.

In this final chapter the results from the three experiments and further analysis of the DFA method are summarised. Finally, the future prospects for use of fractal analysis are discussed and some possible directions for future research are suggested.

8-2. Summary of findings

8-2-1. Experiment One: Acute stress in chickens

Exposing young ISA Brown hens to mildly stressful situations such as placement in a novel arena or a five-minute period of mechanical restraint caused an increase in the complexity (randomness) of vigilance behaviour as measured using DFA. The total duration spent in vigilance was also increased in the novel arena but not following the period of restraint, so in the latter case the DFA revealed an alteration in behavioural organisation under the mild stress that was not seen using standard measures.

Previous applications of fractal analysis to behavioural patterns (Motohashi et al. 1993; Alados et al. 1996; Alados & Weber 1999; Alados & Huffman 2000), found reduced behavioural complexity under conditions that chronically challenged the animal. In contrast, in this experiment, an increased behavioural complexity was found under short-lived conditions that putatively induced fear and an associated mild distress in the animals. This difference matches intuitive expectations as to the effects of chronic and acute challenges on behaviour. The key point is that deviation from normality can occur in either direction.

8-2-2. Experiment Two: Chronic-intermittent stress in chickens

Adult ISA Brown laying hens were repeatedly exposed to numerous varied acute stressors over two ten-day periods. Changes in body weight, food intake and egg weight indicated that the stress group birds were mildly stressed for at least part of the treatment duration. However, corticosterone concentrations were actually larger in the control group following the first period of stressor exposure and the heterophil to lymphocyte (H/L) ratio, a commonly used indicator of stress in poultry, did not differ between the two groups at any time point. Standard behavioural measures also did not differ between the two groups. DFA on either activity or vigilance patterns did not reveal any difference in behaviour between control and stress group birds. There was, however, a highly significant negative relationship between the complexity of activity and the H/L ratio in one of the four observations.

8-2-3. Experiment Three: Chronic stress in pigs

Growing pigs were individually exposed to a chronic stress treatment involving repeated aggressive interactions with an unfamiliar larger pig and additional environmental stressors such as wetting or removal of the substrate, or unavoidable airflow. DFA was applied to various different behavioural categories. The analysis showed that, following the stressor period, the fluctuating pattern of postural activity (i.e. the changes back and forth between standing/walking and sitting/lying) was significantly more structured (less random) in test pigs compared to controls. No detectable differences were found in the total amount of activity or in its circadian pattern. Circulating cortisol levels were also higher in test pigs, compared to controls, over the 24-hour period following stress exposure. For all pigs (i.e. test and control pigs), following the stressor period, cortisol concentration was correlated with the fractal structure of postural activity, such that pigs with higher cortisol values had a more structured behavioural pattern.

The results suggest that the stressor treatment did create a mild chronic stress, as indicated by a hypercortisolaemia and reduced weight gain in the test pigs, relative to controls. The results of the behavioural analysis show that fractal techniques, such as DFA, can be applied to pig behaviour and that they can reveal extra novel information about the structure of an individual's behavioural organisation.

8-2-4. DFA Methodology

In both chicken experiments, neither of the fractal descriptions of vigilance or of activity consistently correlated with the corresponding total behavioural duration. The fractal measures did, therefore, appear to provide a novel descriptor of behavioural organisation. Although, the fractal measure of vigilance behaviour did consistently relate to the frequency of vigilant behaviours, the fractal measure revealed treatment differences in the acute stress experiment that were not revealed by an analysis of the frequency of behavioural events.

In the second chicken experiment the temporal complexity of vigilance behaviour was found to be consistent across repeated observations for individual animals, suggesting that it may represent a consistent behavioural trait of individual animals.

Such individual consistency in behavioural complexity was also found for feeding behaviour in pigs in the third experiment.

Reducing observation length caused the behavioural records to be recorded as being more structured than at the full length. Maintaining observation length but decreasing sampling frequency had the opposite effect, such that records were recorded as being more random than at the highest resolution.

8-3. Directions for future research

8-3-1. Experimental work

This study has shown that fractal analysis can be easily applied to behavioural observations in two farm animal species, the pig and the chicken, in which there are wide ranging welfare concerns.

Rushen (2003) recently noted that many parameters (behavioural and others) have been used as welfare indicators despite a poor understanding of their biological basis. It is important that we do not fall into this trap with fractal analysis. It could be said that fractal analysis provides a fine scale tool with which to describe very subtle alterations in behaviour. Alternatively, a critic could argue that the alterations in behaviour are so small as to be meaningless. It is this point (which may well have some basis) that necessitates careful validation of fractal analysis.

As a first step towards investigating fractal analysis as a welfare assessment tool the work described here perhaps raises more questions than it provides answers. However, new stress assessment tools do not just appear fully formed and ready to use overnight. Proper validation of the methods and investigation of what they mean in terms of an animal's behaviour, as well as what non-stress related factors can influence them, is required. Key requirements for future research are:

1. **Examination of factors that could affect fractal measures of behaviour.**
   The DFA method provides a measure of behavioural organisation that could potentially reveal very subtle alterations in behavioural structure. Even if these behavioural parameters alter in situations of poor welfare, to be able to use them as a measure in welfare assessment it will be necessary to identify in what other situations they change. It is likely that there will be factors that cause fractal

behavioural complexity to alter, yet have no impact on an animal's state of well being. It is possible, indeed likely, that fractal complexity could alter in positive situations just as some physiological indicators, such as glucocorticoids, do.

2. **Relationship between fractal measures of behaviour and stress physiology.**

One key criterion for identifying a behaviour relating to stress is comparison with physiological parameters (Ewbank 1985). The research in this thesis has provided some slight suggestions of correlations between behaviour and physiology: e.g. the correlation between $A\alpha$ and the H/L ratio in one of the observations in Chapter Five and the correlation between $PA\alpha$ and cortisol concentration in Chapter Six. However, both these results could be statistical flukes and in neither case was the experiment ideally set up to specifically test for such correlations (i.e. physiology sampling and behavioural observation were separated in time).

The use of biotelemetry equipment could be particularly useful to investigate correlations between physiological (e.g. heart rate) or neurobiological function (e.g. EEG) and the fractal organisation of behaviour. Indwelling catheters could be used to more closely investigate the relationship between behaviour and physiology.

3. **Exogenous alteration of physiological state.**

Following on from the previous section, one useful way of assessing the relationship between various physiological variables and the fractal complexity of behaviour would be to artificially alter circulating levels of the variables and measure any resulting change in behaviour. For instance, the HPA axis can be affected at various levels by exogenous treatment with CRH, ACTH or glucocorticoids (e.g. Parrott & Vellucci 2000; Puvadolpirod & Thaxton 2000abcde; El-lethey et al. 2001).

4. **Relationship between health and fractal measures of behaviour.**

Fractal analysis potentially provides a tool for identifying subtle alterations that may well occur during states of sub-clinical stress or in the early stages of disease. Behavioural measures are under utilised in studies of disease processes

and their treatment or prevention. Many studies would benefit from a sensitive and non-invasive measure of effect onset or severity. Similarly, studies of conditions that may be chronically painful, such as mastitis in cattle or sheep scab, could benefit from the use of a sensitive behavioural assay.

Experimentally, the non-specific sickness behaviours associated with disease can be elicited through administration of an injection of lipopolysaccharide (LPS) (e.g. Johnson et al. 1993; Johnson & von Borell 1994; Warren et al. 1997; Tilders & Schmidt 1999). Although, larger doses of LPS produce a profound behavioural depression in the hours following injection, a smaller dose could mimic the sub-clinical infections that are undoubtedly rife in both the pig and chicken agricultural populations.

8-3-2. Analysis

DFA is only one of a large array of different fractal analysis techniques that could potentially be applied to behavioural data. When multiple measures are applied to the same data it is commonly found that the different methods may reveal subtly different things about the structure. Alternatively, some methods may be equivalent in which case it would be sensible to discard the more complex methods.

Future work could involve comparisons of the DFA method with other analysis measures, such as spectral analysis or other fractal measures such as rescaled range analysis. The approximate entropy measure (Pincus 1991; Pincus & Goldberger 1994), whilst not a fractal measures, appears to provide information of a similar sort to that accessed by fractal analysis. The extent to which these different measures are equivalent or alternatively provide subtly different forms of information about temporal organisation needs to be investigated.

The use of automated collection of behavioural data may be especially important for examination of fractal analysis methodologies. These tools can provide an amount of data that would be impossible to collect using direct observation. Obviously the use of automated collection devices does place a limit on the types of behavioural data that can be collected, but since fractal analysis methods such as DFA are more suited to analyses of simple behavioural transitions between two clear and repeating states than to analysis of rare or subtle behaviours

the sort of information that can be gained from automated sampling may be ideally suited to DFA.

8-3-3. Potential use of fractal analysis in welfare studies

Although the work presented here has not revealed a definite relationship between stress states and altered behavioural complexity the results are still encouraging. How then might fractal analysis be used in welfare research? In acute stress situations it could provide- more precise quantification of the lasting impact of an acute challenge. The ability of animals to adapt to acute challenge is an important determinant of welfare and one that may be altered when environmental conditions and/or the background genome lead to exaggerated fearfulness, anxiety or hyperexcitabilty (Jones 1996; Rosen & Schulkin 1998; Boissy et al. 2001). In this sense altered fractal complexity could be used in welfare research as an indicator variable (Fayers & Hand 2002).

Previous work (Alados et al. 1996; Alados & Weber 1999; Alados & Huffman 2000) suggested that reduced behavioural complexity might correlate with states of increased allostatic load (McEwen & Wingfield 2003) or pre-pathological/sub-clinical stress states (Moberg 2000) where an animal continues to function ostensibly as normal yet is impaired and vulnerable to further challenge. Clear behavioural indicators of such a state are not currently available but would be of great benefit in welfare assessment.

Alternatively, if poor welfare results from lowered behavioural complexity reduced behavioural complexity during chronic stress could actually be viewed as a causal variable (Fayers & Hand 2002). Physiological studies suggest that complexity/irregularity is healthy as it allows body systems to readily adapt to challenge and that with age or disease comes a loss of complexity in biological functioning resulting in a decreased ability to cope (Goldberger 2002ab). Alados and Weber (1999) also suggest that a degree of complexity in behaviour patterns is beneficial in dealing with events in the environment and that decreased behavioural complexity could leave animals vulnerable to challenge. Whether this is the case remains to be established. In this context, the influence of environmental rearing conditions of the fractal complexity of behaviour patterns needs to be established. Inglis (2000) suggests that, in a heterogeneous changing environment behavioural

variability will be favoured over regularity because animals can never be absolutely certain that the cognitive expectations they have formed about the environment will be entirely correct. A corollary of this is that behaviour variability may decrease when animals are kept in bland environments.

Spatial analyses such as those used by Paulus and Geyer (1991, 1993) might provide a useful additional measure of open field behaviour, beyond those currently used. These studies have shown that a spatial fractal measure is independent of total activity in rodents and that spatial and temporal fractal measures of behaviour can identify different alterations caused by drug treatments.

Fractal analysis might also play a role in the investigation of abnormal behaviours. For example, might a decrease in behavioural complexity be a precursor to stereotypic behaviour or, more generally, might fractal analysis provide novel measurements of stereotypy? For example, Paulus and co-workers (1999b) found that compared to controls schizophrenic humans showed a larger and longer lasting dependence of current behaviour on previous behaviour using a fractal measure. Behavioural tests relating to such perseverance in behaviour are being investigated in animal behaviour studies relating to stereotypic behaviour (e.g. Garner & Mason 2002). An alteration in fractal structure could provide a measure of the abnormal expression of normal behaviours.

8-4. Conclusion

Fractal analysis is used to describe and measure complexity in a variety of different fields and is thought to reveal otherwise hidden information about organisational properties of complex systems (Peng et al. 2000). These features are rarely considered in behavioural research and it is this fact that makes fractal analysis a potentially useful methodology. In many cases, fractal analysis enables assessment of when complex organisational properties change or how they compare in different situations. Fractal analysis can therefore add to current analyses by revealing previously 'hidden' information about behavioural organisation. Calculation of a fractal exponent, as a description of statistical or sequential properties of behavioural organisation, provides additional information above and beyond that provided by simpler analyses of mean frequencies and durations of behaviour.

A small number of studies, including the work presented here, suggest that fractal measures can reveal alterations in behaviour relating to well being when standard measures do not. Although the significance of this for welfare assessment remains to be fully understood it is still possible to say that the ability to extract more information from our data can never be detrimental. Indeed, it is more likely that fractal analysis could add to the range of tools used within welfare assessment by providing the opportunity to assess stress in a non-invasive way using data that is already routinely collected.

Although collectively, the findings tentatively suggest that fractal analysis could play a useful role in welfare assessment, if any of the available fractal measures are to prove useful, more detailed examination of them will be necessary. Fractal measures need to be stringently validated against currently available indices of poor welfare, such as physiological markers of stress, metabolic and immunological parameters.

The work presented here has shown that novel measures of the temporal organisation of various behavioural patterns can be measured in chickens and pigs. In both species it was shown that these measures can vary between groups of individuals when more standard measure of behaviour do not. There is, however, a wide gap between these facts and being in a position to say that fractal analysis is an indicator of stress or any other aspect of decreased animal welfare. The development of a measure of stress is a slow process and this work is a starting point for further investigations of fractal analysis as a tool in welfare assessment.

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Applied Animal Behaviour Science 83 (2003) 125–139
APPLIED ANIMAL BEHAVIOUR SCIENCE
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Detrended fluctuation analysis of behavioural responses to mild acute stressors in domestic hens

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Accepted 8 April 2003

Abstract

Fractal analysis provides a novel measure of behavioural complexity and has previously revealed subtle alterations in behaviour under biologically costly conditions, such as parasitism or disease. The analysis is based upon the temporal pattern of behaviour that, although rarely considered in behavioural studies, may provide information in addition to standard measures of duration and frequency. Such information could be useful in assessing the welfare of confined animals.

Using ISA Brown pullets, we wished to test the hypothesis that fractal analysis reveals novel behavioural alterations during stress. The behaviour of undisturbed birds in their home pen was compared to the behaviour of the same birds: (1) in a novel arena, (2) in their home pen following blood withdrawal and (3) in their home pen following 5 min of mechanical restraint plus blood withdrawal. Detrended fluctuation analysis (DFA), which calculates fractal complexity measures for time series data, was applied to sequences of vigilance behaviour and walking. These two behavioural parameters where chosen because they are relatively simple to measure and might be expected to alter under stress.

When compared to home pen behaviour, complexity in vigilance behaviour increased in the novel arena ($P < 0.001$) and following restraint and blood sampling ($P < 0.05$) but was unaltered following blood withdrawal only ($P = 0.36$). Total time spent vigilant was increased in the novel arena ($P = 0.001$) but not following restraint ($P = 0.45$) or blood withdrawal ($P = 0.11$). The complexity of walking patterns and the total time spent walking were similar in all situations.

In conclusion, DFA provides a novel measure of temporal behavioural complexity in chickens. In contrast to studies of chronic situations in other animals, acute stress caused an increase in behavioural complexity in the present experiment. This increased complexity occurred independently of changes

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0168-1591/$ – see front matter
doi:10.1016/S0168-1591(03)00115-1

in the duration of behaviour suggesting that DFA can reveal more subtle changes in behavioural organisation during stress. If such behavioural alteration represents a non-specific stress response this methodology could allow objective comparisons of different stressors to be made.

Keywords: Chickens; Stress; Fractals; Behavioural complexity; Detrended fluctuation analysis

1. Introduction

Behavioural analysis has an important role in the assessment of stress in animals. A concern though is that behavioural responses may be highly specific to individual stressors, particularly in the case of acute stressors (Dawkins, 1999; Rushen, 2000). This may limit comparison of different stressful stimuli because it is not possible to reliably compare the severity of selected stressors if they elicit responses that vary qualitatively rather than quantitatively (Rushen, 2000). Therefore, in order to judge the relative severity of different events or situations it is necessary to be able to measure stress effects on a single non-specific scale. One parameter that might potentially meet these requirements is behavioural complexity. Recently, fractal analysis has emerged as a potentially useful measure of behavioural complexity.

The original concept of fractals arose from attempts to mathematically characterise complex patterns in nature (Mandelbrot, 1977). A key feature of fractal patterns is the statistical property of scaling. In this context scaling means that the properties of the structure or process vary with the scale or resolution of analysis. For instance, in geometry, measuring a very complex object at a smaller scale means more of the complex fine detail is revealed and the measured size is larger. For a fractal object or process, a power law describes the relationship between measured size and measurement scale. In fractal analysis, the degree of scaling is measured and assigned a parameter, typically called the fractal dimension, which is seen as a measure of complexity. Since fractals can be used to describe complex systems, they can therefore also identify when the properties of those systems change. For instance, fractal analysis of heart rate variability can differentiate between patients on the basis of previous heart conditions (Saermark et al., 2000) and may prove useful as a predictor of future risk of heart problems (Ho et al., 1997).

Fractal analysis of animal behaviour has been proposed as an indicator of well being in various species (Alados et al., 1996; Alados and Weber, 1999; Alados and Huffman, 2000). Pregnant or parasitised Spanish ibex were found to have a less complex pattern of vigilance and feeding behaviours than controls (Alados et al., 1996). Interestingly, despite the fact that these behavioural patterns were significantly altered standard behavioural measures did not differ, e.g. pregnant animals spent as long feeding and showed as many head-lifts as non-pregnant animals. More recently, lowered complexity in the reproductive behaviour of fathead minnows exposed to lead (Alados and Weber, 1999) and in the social behaviour of diseased chimpanzees (Alados and Huffman, 2000) has been reported. Thus, fractal analysis may reveal 'hidden information' (*sensu* Peng et al., 2000) about the organisation of behaviour beyond that extracted using conventional behavioural analyses, which are often limited to measures of mean duration or frequency of particular behaviours. These

later studies (Alados and Weber, 1999; Alados and Huffman, 2000) use a form of fractal analysis called detrended fluctuation analysis (DFA), which is also applied here.

In the present experiment, we asked whether a fractal analysis technique could be used to identify general properties of behavioural complexity and if these measures might alter in mildly stressful conditions. DFA was applied to the behaviour patterns of chickens that remained undisturbed in their home pen (HP), or that were exposed to the stress of blood sampling (BS), mechanical restraint (R) plus BS or placement in a novel arena (NA). Mechanical R using a crush cage is a standard experimental stressor in poultry (Satterlee and Johnson, 1988; Jones et al., 1994). Exposing animals to an unfamiliar environment (usually referred to as an open field) is a commonly used test of fear and anxiety. Both the novelty value and lack of shelter within the NA are likely to cause fear, which is a potent stressor (Jones, 1996). The BS and R procedures used here also involve handling and transient social isolation, both of which are likely to be stressful.

2. Animals, materials and methods

2.1. Animals and housing

Forty-eight ISA Brown hens were reared to 11 weeks of age in cages and then transferred to floor pens (105 cm × 100 cm) bedded with wood shavings, 1 week before the first observation. The birds were housed in pairs in the cages and these pairs were maintained in the floor pens. The mean weight of these birds was 1.18 kg. One bird from each pair was randomly designated as the test bird, with the other acting as a companion. The birds were identified by leg rings. After transfer to floor pens all birds were food deprived for 5 h each day (either from 8:00 to 13:00, 10:30 to 15:30 or 12:00 to 17:00 h, to tie in with experimental treatments). The food deprivation period was used to increase motivation to feed, to ensure a period of active behaviour during the subsequent observations. Water was always available. Birds were kept on a 14 h:10 h light/dark regime, with lights on from 8:00 to 22:00 h. On test days, when behaviour was recorded, 100 g of pelleted food was scattered into the pen after 4 h of deprivation, at which point the observation began.

2.2. Test situations

2.2.1. Home pen

Three repeated observations (HP1, HP2 and HP3) of the behaviour of birds that remained undisturbed in their HP were made.

2.2.2. Novel arena

The test and companion birds were transferred to a novel test arena. This involved carrying them in a wire holding cage approximately 100 m down a corridor to another room. Three NAs were used in different soundproof rooms (floor dimensions: 120 cm × 120 cm (two arenas), or 140 cm × 140 cm) with three pairs of birds being tested simultaneously. The walls of the arena were made of unpainted plywood, wood shavings were spread on the

floor and a wire mesh roof prevented escape. When both birds had been placed in the test arena, food was scattered and the observation began.

2.2.3. Blood sampling

The test bird was removed from the HP to an adjacent room and restrained manually while 2 ml of blood was removed from the brachial vein. The bird was then returned to the HP, food was scattered within the pen and the observation began.

2.2.4. Restraint

The test bird was removed from the HP and restrained for 5 min in a mechanical crush cage. The crush cage consisted of a box made of stainless steel mesh with a moveable, solid, internal partition. The bird was placed in one end of the cage and the partition was moved up against the bird until it was unable to turn around. This degree of R allowed the bird some forward–backward movement and did not impair respiration. The crush cage was in the same room as the other birds but the test bird was physically and visually isolated from its companion. After this 5-min period, the bird was removed from the cage and a 2 ml blood sample was taken. The bird was then returned to the HP, food was scattered and the observation began.

2.3. Observation details

The bird's behaviour was recorded onto videotape in each test situation, using a camera located either at the front of their HP or above the NA. During the observation period the experimenter did not enter the room. A simple ethogram (Table 1) consisting of events and mutually exclusive states was used to classify the test birds' behaviour. The timing of behavioural events and transitions between states was determined from the video recordings using the Keytime computer programme (Deag, 1993). The observation length was 3072 s (51 min and 12 s) to fit in with the DFA analysis (see Section 2.4). Each bird was observed a total of six times. The birds were divided into two batches that differed only in the order they received the BS or R treatments. In the first batch, the observation order was: HP1, NA, BS, HP2, R, HP3. In the second batch the observation order was: HP1, NA, R, HP2, BS, HP3. There was an interval of 3 or 4 days between each observation on the same bird. Each set of observations took 3 days (eight birds were observed each day; three at 12:00 h, three at 14:30 h and two at 16:00 h). Each bird was observed at the same time of day in each test situation.

Some observations were not recorded onto computer because of major disturbances during recording that were beyond the experimenter's control. There were also some instances when the companion obscured the test bird for lengthy periods and these were also discarded. The resulting samples sizes were therefore: $n = 24$ for the HP observations, $n = 23$ for the NA observation, $n = 16$ for the observations following BS only treatment and $n = 17$ for observations following the BS and R treatment.

2.4. Detrended fluctuation analysis

The method used was based on previous behavioural work (Alados and Weber, 1999; Alados and Huffman, 2000) and is also described by Peng et al. (2000). The analysis was

Table 1
Ethogram definitions, based on references in the literature (Kruijt, 1964; Black and Hughes, 1974; Wood-Gush, 1989)

	Definition
States	
Standing head up	Bird is upright with sternum clear off ground, stationary on one or both legs. Head is above horizontal midline of body, back of head higher than line of back.
Standing head down	Bird is upright with sternum clear off ground, stationary on one or both legs. Head is below horizontal midline of body, back of head below line of back.
Standing preen	Bird is upright with sternum clear off ground, stationary on one or both legs. Bird directs attention with beak (pecking, stroking, combing or nibbling) towards body and feathers.
Walking head up	Bird takes at least two consecutive steps with head up.
Walking head down	Bird takes at least two consecutive steps with head down.
Sitting head up	Bird's body resting on ground, with head up.
Sitting head down	Bird's body resting on ground, with head down.
Sitting preen	Bird's body resting on ground, preening.
Dustbathe	Bird engages in dustbathing behaviour, kicking litter onto body and wiggling body about in dust. Feathers ruffled.
Events	
Wing flap	Agitated, repeated movement of wings. Bird may or may not be moving.
Wing stretch	Either bilateral or unilateral, upward/sideways extension of wing(s).
Test peck	Test birds directs peck towards companion.
Companion peck	Companion directs peck towards test bird.
Drink	Bird pecks at water drinker (suspended from ceiling).
Body shake	Feathers raised and body shakes. Includes tail shaking.
Head shake	Head is moved rapidly from side-to-side.
Ground scratch	Bird scratches in sawdust making backward strokes with leg: typically body moves down and bird moves forward then back.

applied to the pattern of vigilance behaviour, as crudely measured by head lifting, and the pattern of walking. Here, we will describe the method for analysing the fluctuation between vigilance and non-vigilance only but the same process was also applied to the walking pattern. The behavioural data was recorded in the form of a time series of events and mutually exclusive behavioural/postural states. Events and the times they occurred were discarded from this record leaving a series of times representing changes between the mutually exclusive behavioural states. For DFA purposes these were combined into binary states, i.e. standing head up, sitting head up and walking head up are all classified as vigilant while all the other behaviours are non-vigilant. Behaviour was then classified as vigilant or non-vigilant at half-second time points (Eq. (1)).

$$x_i = \begin{cases} 1, & \text{if bird is vigilant} \\ -1, & \text{if bird is otherwise} \end{cases} \quad (1)$$

A 'cumulative vigilance score' (y) was then created by adding 1 to the variable at each time point (i) if the behaviour was vigilant, and subtracting 1 if it was non-vigilant (Eq. (2))

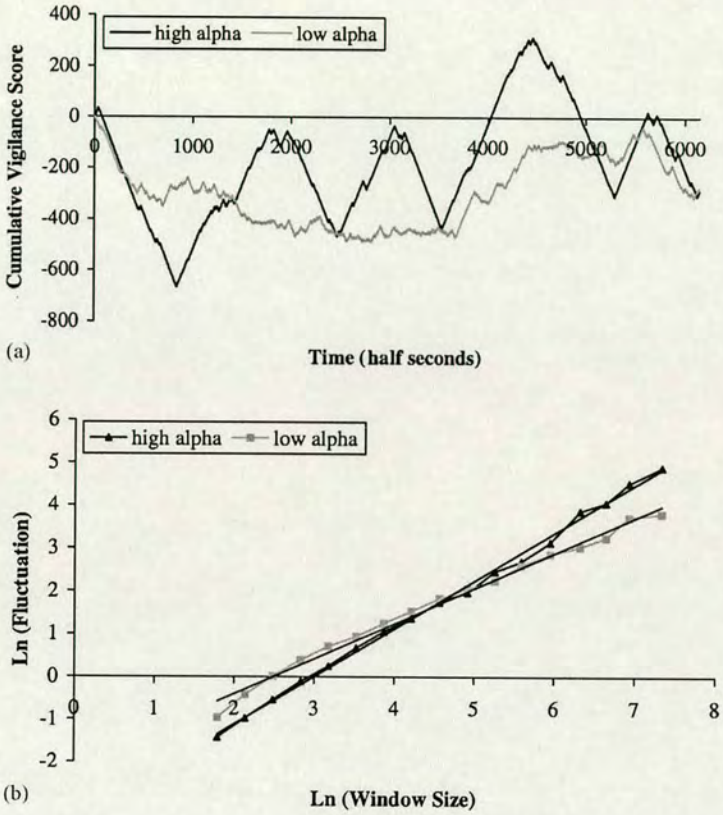

Fig. 1. Extremes of fractal complexity in the vigilance data set. (a) The two original time series for the birds with either the highest (black line) or lowest (grey line) DFA α-values (from all observations) representing the extremes of high and low long-range autocorrelation, respectively. (b) Fluctuation values against window size for the same two series, plotted on a double log scale. The series (black triangles) with the highest α-value (lowest behavioural complexity) is characterised by the equation $y = 1.12x-3.348$ (black line), $R^2 = 0.997$. The series (grey squares) with the lowest α-value (greatest complexity) is characterised by the equation $y = 0.82x-2.045$ (grey line), $R^2 = 0.99$.

(see Fig. 1a for an example).

$$y_i = \sum_{j=1}^{i} x_j \tag{2}$$

The observation length was set at 3072 s (51 min and 12 s). At a resolution of half a second, this yielded a time series of 6144 data points. The continuous time series (y_i) was subdivided up into m non-overlapping 'windows', within each of which a regression line was fitted (Eq. (3)). The value of m followed the sequence: 2^2, $2^{2.5}$, 2^3, $2^{3.5}$, ..., 2^{10} (rounded to the nearest integer; 4, 6, 8, 11, ..., 1024). The size of each window was represented by n and decreased from 1536 at $m = 4$, down to only six data points (representing 3 s of behaviour) at $m = 1024$. Since n was not necessarily always an integer value the regression lines were

fitted into windows according to equation four below, where k, rounded down to the nearest integer, represented the particular window number (1–m). The degree of fluctuation (F: the root mean square of the errors), at varying values of n, was then calculated (Eq. (5)).

$$\hat{y}_i^n = a_{k(i,n)} + b_{k(i,n)}i \tag{3}$$

$$k(i, n) = \left(\frac{i-1}{n}\right) + 1 \tag{4}$$

$$F(n) = \sqrt{\frac{1}{6144}\sum_{i=1}^{6144}(y_i - \hat{y}_i^n)^2} \tag{5}$$

Once the calculation of F at different window sizes was complete, window size was plotted against fluctuation value on a log–log scale (Eq. (6)) (see Fig. 1b for an example). Typically, the fluctuation value was much larger at large window sizes. As window size decreases the regression lines become more closely fitted to the data and the measure of fluctuation decreases. A straight line in the log–log plot indicates that a power law relates window size and fluctuation, with the slope of the log–log plot equal to the power law exponent, α (Eq. (7)).

$$\ln(F(n)) = \ln(a) + \alpha \ln(n) \tag{6}$$

$$F(n) = an^{\alpha} \tag{7}$$

The α-value (log–log plot slope/power law exponent) relates to the autocorrelation structure of the time series. In this case, if it equals 0.5 the series is said to be uncorrelated (random), while if it is greater than 0.5 the series is said to show long-range autocorrelation. This means that on-going behaviour is influenced by what has occurred in the past. Note that in DFA, the α exponent is inversely related to a typical fractal dimension, so in this case the value increases with increasing regularity (decreasing complexity) in the time series.

2.5. Statistical analysis

Total behavioural duration (for each state) or frequency (for events) was calculated for each observation in Keytime. For each parameter (either behavioural durations or frequencies, or DFA exponents), a single HP value (HP: the mean of all three repeated HP observations) was calculated for each bird. This was then subtracted from the NA, BS or the R plus BS values (i.e. each bird was used as its own control). One-sample t-tests were used to determine if the resulting value differed from 0.

3. Results

3.1. Behaviour—standard measures of duration and frequency

Within the HP, on average 47.8% of the observation period was spent in vigilant postures/behaviours (see Table 2 for the descriptive statistics for vigilance and walking and Table 3 for values for the individual behavioural states). In the NA, there was a significant

Table 2
Descriptive statistics (means + S.E.) for vigilance and walking recorded during the home pen observations (HP, $n = 24$), and the mean differences between those values and those recorded in the other three treatments: novel arena (NA, $n = 23$), blood sampling (BS, $n = 16$) and restraint plus blood sampling (R + BS, $n = 17$)

	Behavioural category					
	Vigilance			Walking		
	Duration (s)	Total number of bouts	Mean length (s)	Duration (s)	Total number of bouts	Mean length (s)
HP	1469.3 (54.1)	154.9 (4.9)	10 (0.65)	128.9 (14.3)	75.3 (6.8)	1.6 (0.04)
NA-HP	416 (111.1) $t = 3.74$, $P = 0.001$	−4.46 (8.3) $t = -0.54$, $P = 0.6$	2.76 (1.3) $t = 2.11$, $P = 0.046$	45.2 (32.8) $t = 1.38$, $P = 0.18$	9 (11.2) $t = 0.8$, $P = 0.43$	0.32 (0.1) $t = 3.21$, $P = 0.004$
BS-HP	153 (90) $t = 1.7$, $P = 0.11$	3.55 (5.3) $t = 0.67$, $P = 0.51$	0.41 (0.77) $t = 0.54$, $P = 0.6$	9.2 (28) $t = 0.33$, $P = 0.75$	4.8 (13.6) $t = 0.35$, $P = 0.73$	−0.05 (0.06) $t = -0.83$, $P = 0.42$
R + BS-HP	94.5 (121.6) $t = 0.78$, $P = 0.45$	16.32 (9.1) $t = 1.79$, $P = 0.09$	−0.36 (1.8) $t = -0.2$, $P = 0.85$	−22.7 (22) $t = -1.03$, $P = 0.32$	−10 (13.4) $t = -0.75$, $P = 0.46$	−0.14 (0.09) $t = -1.51$, $P = 0.15$

Table 3
The durations (s: means + S.E.) of behavioural states recorded during the home pen observations (HP, $n = 24$), and the mean differences between those values and those recorded in the other three treatments: novel arena (NA, $n = 23$), blood sampling (BS, $n = 16$) and restraint plus blood sampling (R + BS, $n = 17$)

	Behavioural State								
	Standing head up	Standing head down	Standing preen	Walking head up	Walking head down	Sitting head up	Sitting head down	Sitting preen	Dustbathe
HP	1024.5 (53.1)	984.8 (71.3)	220.8 (29)	96.5 (13.9)	32.45 (5.21)	289.5 (35.4)	129 (22.5)	235.6 (34.8)	58.9 (27.5)
NA-HP	340.4 (89.9) $t = 3.79$, $P = 0.001$	63.4 (120.2) $t = 0.53$, $P = 0.6$	−176.5 (32.9) $t = -5.37$, $P < 0.001$	21.4 (31.8) $t = 0.67$, $P = 0.51$	23.8 (9.3) $t = 2.56$, $P = 0.018$	113.8 (127.4) $t = 0.89$, $P = 0.38$	−108.7 (23.9) $t = -4.56$, $P = 0.002$	−218 (37.8) $t = -5.76$, $P < 0.001$	−59.6 (28.7) $t = -2.08$, $P = 0.05$
BS-HP	76.3 (85.1) $t = 0.90$, $P = 0.38$	−244.6 (128.1) $t = -1.91$, $P = 0.075$	−27.9 (41.8) $t = -0.67$, $P = 0.51$	9.1 (24.9) $t = 0.36$, $P = 0.72$	0.1 (5.5) $t = 0.02$, $P = 0.98$	127.5 (79.6) $t = 1.6$, $P = 0.13$	38.3 (53.3) $t = 0.72$, $P = 0.48$	73.5 (56) $t = 1.31$, $P = 0.21$	−59.9 (39.9) $t = -1.5$, $P = 0.15$
R + BS-HP	78.6 (115.7) $t = 0.68$, $P = 0.51$	−237.1 (120.5) $t = -1.97$, $P = 0.067$	121.1 (64) $t = 1.89$, $P = 0.077$	−11.6 (20.1) $t = -0.58$, $P = 0.57$	−11.1 (7.4) $t = -1.49$, $P = 0.16$	42.8 (93.5) $t = 0.46$, $P = 0.65$	34.1 (47) $t = 0.73$, $P = 0.48$	−5 (44.1) $t = -0.11$, $P = 0.91$	−15.3 (56.7) $t = -0.27$, $P = 0.79$

increase in the total time spent vigilant, principally due to an increase in standing with head up and to decreased standing preening, sitting preening and sitting with head down. There was no detectable effect of the BS or the R and BS procedures on vigilance when the bird was returned to the HP.

The average occurrence of vigilant states in the HP was not significantly altered in the NA or following BS, although there was a trend towards a slight increase following R. The mean bout length of vigilance was significantly increased in the NA but not following BS or R plus BS. The total duration of vigilance in the HP was negatively correlated with the change in vigilance in the NA ($r = 0.66$, $P < 0.01$), such that birds with a low level of HP vigilance showed a larger change than those with a high level of HP vigilance.

There was no alteration in the total time spent walking or the number of walking bouts in the NA, or following BS or R plus BS compared to the HP observations. There was a small yet significant increase in the mean duration of walking in the NA, but no change following BS or R plus BS compared to the undistributed HP observations.

Scratching, drinking and head shaking were the most common events in the HP (Table 4). In the NA, the frequencies of stretching, test pecking and scratching were all reduced. Following BS, stretching was also reduced and there were trends towards decreased flapping and scratching. Following the R plus BS procedure, there were reductions in stretching and drinking and trends towards decreased flapping and scratching and increased head shaking.

3.2. Detrended fluctuation analysis

The original time series for the smallest and largest α exponents (over all observations) are plotted (Fig. 1a) to illustrate extremes of complexity and regularity. Despite the large difference in the fractal structure of their vigilance behaviour, these two birds showed almost exactly the same total amount of vigilance over the observation period: the bird with the highest α-value (indicating low behavioural complexity) spent 24 min and 25 s vigilant, while the bird with the lowest α-value (most random vigilance pattern) spent 24 min and 26 s vigilant. Fig. 1b shows the double log plots of fluctuation against window size for these two series. For all the regressions on the log–log plots, the mean t-value was 56.18 (S.D. = 15.01). The t-value at a significance level of $P = 0.001$ would be 2.947 and all the regression lines exceeded this value thus indicating high goodness-of-fit.

The mean HP α-value of 0.98 (S.D. = 0.042, range = 0.90–1.08) was significantly reduced (indicating increased complexity) in the NA (NA-HP, mean = −0.04, $t = -4.44$, d.f. = 22, $P < 0.001$) and following R (R-HP, mean = −0.02, $t = -2.42$, d.f. = 16, $P < 0.05$) but it was not significantly altered following BS (BS-HP, mean = −0.009, $t = -0.94$, d.f. = 15, $P = 0.36$). There was no effect of the order of BS and R plus BS, nor was there an interaction between treatment and order (GLM: order effect, $F_{1,32} = 0.00$, $P = 0.99$; interaction, $F_{1,32} = 0.98$, $P = 0.33$).

The DFA on walking pattern produced a mean HP α-value of 0.70 (S.D. = 0.051, range = 0.58–0.81). This did not alter significantly in the NA (NA-HP, mean = −0.01, $t = -0.66$, d.f. = 22, $P = 0.52$), or after BS (BS-HP, mean = 0.004, $t = 0.28$, d.f. = 15, $P = 0.79$) or R plus BS (R + BS-HP, mean = 0.004, $t = 0.38$, d.f. = 16, $P = 0.71$).

The DFA values for vigilance did not correlate with the total duration of vigilance shown (HP observations only: $r = 0.16$, $n = 24$, $P = 0.45$; all observations: $r = 0.086$, $n = 80$,

Table 4
The frequencies (means + S.E.) of behavioural events recorded during the home pen observations (HP, $n = 24$), and the mean differences between those values and those recorded in the other three treatments: novel arena (NA, $n = 23$), blood sampling (BS, $n = 16$) and restraint plus blood sampling (R + BS, $n = 17$)

	Behavioural Event							
	Wing flap	Wing stretch	Test peck	Companion peck	Drink	Body shake	Head shake	Ground scratch
HP	1.8 (0.26)	2.7 (0.29)	7.6 (2.46)	2.6 (1)	24 (5.35)	1.3 (0.17)	11 (2.12)	27.3 (5.1)
NA-HP	−0.23 (0.4) $t = -0.58$, $P = 0.57$	−1.4 (0.46) $t = -3.04$, $P < 0.01$	−5.93 (2.59) $t = -2.29$, $P = 0.03$	1.65 (1.55) $t = 1.06$, $P = 0.3$		0.5 (0.3) $t = 1.66$, $P = 0.11$	−4.56 (2.72) $t = 1.68$, $P = 0.11$	−21.08 (4.99) $t = -4.22$, $P < 0.001$
BS-HP	−0.83 (0.43) $t = -1.94$, $P = 0.07$	−1.83 (0.48) $t = -3.84$, $P < 0.01$	−1.55 (2.42) $t = -0.64$, $P = 0.53$	−2.15 (1.67) $t = -1.28$, $P = 0.22$	−5.58 (4.94) $t = -1.13$, $P = 0.28$	0.15 (0.28) $t = 0.52$, $P = 0.61$	5.85 (4.85) $t = 1.21$, $P = 0.25$	−12.64 (6.75) $t = -1.87$, $P = 0.08$
R + BS-HP	−0.61 (0.32) $t = -1.89$, $P = 0.08$	−1.98 (0.47) $t = -4.2$, $P < 0.001$	−2.05 (3.98) $t = -0.51$, $P = 0.61$	2.61 (3.12) $t = 0.84$, $P = 0.41$	−15.48 (5.85) $t = -2.65$, $P = 0.02$	0.24 (0.42) $t = 0.56$, $P = 0.59$	9.98 (5.77) $t = 1.73$, $P = 0.10$	−8.43 (4.28) $t = -1.97$, $P = 0.07$

Note: drinking was not possible in the novel arena.

$P = 0.45$). This suggests that the complexity of the vigilance pattern is not simply a function of the total duration of vigilance shown. However, for the walking record there was a strong correlation between the DFA value and the total duration of walking (HP observations: $r = 0.75$, $n = 24$, $P < 0.001$; all observations: $r = 0.55$, $n = 80$, $P < 0.001$).

4. Discussion

The pattern of fluctuation between vigilant and non-vigilant behaviours and between walking and inactivity showed long-range autocorrelation, such as that found in minnow reproductive behaviour and chimpanzee social behaviour (Alados and Weber, 1999; Alados and Huffman, 2000). This means that the behavioural patterns are persistent from moment to moment and that they occur non-randomly. In the case of vigilance, the complexity of the fluctuation pattern increased in the mildly stressful situation of placement in a NA or after R and BS, while BS alone caused no change. The total time spent vigilant also increased in the NA but not in the other situations (Table 2). This indicates that the behavioural pattern is qualitatively but not quantitatively altered following R. DFA therefore reveals information about the nature of behavioural expression, which can alter independently of the total amount of behaviour shown in any given period. This is well illustrated by the plots from two birds showing extremes of complexity but similar final cumulative vigilance scores (Fig. 1a), indicating very similar total duration of vigilance. This suggests that using DFA in addition to traditional analyses can provide valuable additional information about behavioural organisation. Furthermore, the fact that the DFA method can be applied to simple behavioural transitions means that subjective interpretation of behaviour is reduced to a minimum.

In contrast to the alterations seen in vigilance organisation, the fractal structure of the temporal pattern of walking did not alter in any of the observation situations when compared to undisturbed HP observations. It could well be that there are specific reasons why vigilance should change when other behavioural systems do not. The small yet significant shift towards a random fluctuation pattern in the NA and following R compared to that shown in the HP, could represent an adaptation to perceived threat; a random vigilance pattern being supposedly more difficult for a watching predator to 'work out' (Pulliam, 1973). However, it is debatable whether such a small change in behaviour could be viewed as an adaptation. In threatening situations, the most adaptive response would appear to be an increase in total vigilance—as was apparent in the NA. Alternatively, the relatively small amount of walking shown by the birds in their small pens may have decreased the chances of observing a treatment difference. The only alteration seen in walking was a small increase in the mean length of each walking bout in the NA, which could simply reflect the greater space available in the arena compared to the HP. Walking was much more randomly organised than vigilance (as indicated by the lower α-values) and this may reflect a genuine difference in the organisation of walking and vigilance. The difference could, however, also be due to an artefact of the method—walking may be recorded as being randomly organised purely because so little walking was seen. This possibility is partially supported by the fact that the α-value was significantly correlated to the total duration of walking (i.e. birds that spent longer walking had a less random walking pattern). This may mean that, in this experiment, the fractal description of walking is a less reliable measure of behaviour.

It could be argued that, since there were alterations in other behavioural measures in the test situations (Table 4), DFA does not provide any extra information. Exposure to some or all of the stressful treatments reduced the frequencies of stretching, a low priority 'comfort' behaviour (Black and Hughes, 1974), ground scratching and drinking. However, since there is no guarantee that these particular behaviours will occur in any given short observation they are considered unlikely to represent reliable indicators of stress. Furthermore, although the HP, BS and R plus BS treatments provided a putative gradation of stressful stimulation, observed alterations in stretching and scratching did not differentiate between them.

In contrast to previous applications of fractal analysis to behavioural patterns (Alados et al., 1996; Alados and Weber, 1999; Alados and Huffman, 2000), where stress reduced behavioural complexity, we found an increased behavioural complexity, indicating a move towards a more random pattern. This apparent inconsistency probably reflects differences in the nature and duration of the stressors involved. On the one hand, Alados and her colleagues have studied more chronic situations (pregnancy, parasitic load, disease and lead exposure) that impose an energetic cost on the animals but may not necessarily involve any negative mental states such as fear or distress. Conversely, the treatments used in the present study were short-lived and putatively induced fear and an associated mild distress in the animals. In this case, the increased complexity seen in behaviour might be interpreted in terms of a more active response. It is not yet known whether this effect (increased complexity) is peculiar to the particular stressors used in our experiment or to acute stressors in general.

The stressors used here are considered to be relatively mild and not to represent a major welfare concern in themselves. Despite this, our results have important implications for animal welfare science. Although many of the welfare insults associated with housing systems are chronic in nature their impact on an animal's ability to cope with short-term stressors is important (e.g. Boissy et al., 2001). For instance, chronic elevation of plasma corticosterone can increase underlying fearfulness, an increased readiness to respond can reduce the response threshold as well as result in exaggerated responses, and sensitisation of the stress response is thought to contribute to the development of pathological anxiety, hyperexcitabilty and abnormal behaviours (Rosen and Schulkin, 1998; Jones et al., 2000). Thus, increased reactivity to acute stressors may indicate the experience of negative mental states that in turn represent a welfare concern. In addition to this, repeated or exaggerated stress responses themselves may have a physically or cognitively debilitating effect on an animal (e.g. through the long-term effects of increased glucocorticoid levels and 'allostatic load': McEwen and Stellar, 1993; de Kloet et al., 1998).

5. Conclusion

As noted in Section 1, to be able to compare different stressors or environments it is necessary to measure responses on a single non-specific scale. Our results provide preliminary evidence that a fractal analysis methodology could provide such a scale, allowing general statements about the organisation of different behavioural patterns to be made.

The present results show that novel aspects of poultry behaviour can be measured using the DFA method. That the pattern of fluctuation between vigilant and non-vigilant behaviours altered under mildly stressful or fear inducing situations indicates that this method may

have a promising role as a non-invasive measure in overall assessments of welfare. First though, the effects of other changes in the environment (e.g. group size, light levels) or the state of the birds (e.g. hunger, oviposition) that could alter behavioural complexity need to be investigated.

Acknowledgements

We gratefully acknowledge the funding provided by an Animal Welfare Research Training Scholarship to KMDR, from the Universities Federation for Animal Welfare (UFAW). We would also like to thank Nancy Coerse and Anita Rennie for help with experimental procedures, Dave Allcroft for advice on Fortran programming and two anonymous referees for their helpful comments on the manuscript.

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